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THE PURIFICATION OF ADRENAL EXTRACTS AND ISOLATION OF AN ACTIVATOR OF MALE SEX HORMONES[1]

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Extracts containing the life-maintaining hormone of the adrenal cortex can be obtained by various methods. The solubility of the hormone has been found similar to that of the specific sex hormones, and the isolation procedures are similar. One of these methods utilized by Swingle and Pfiffner (1931) has been found reproducible by many investigators. A modified method was published from this laboratory a few years ago (Britton and Silvette, 1931); but the results with these extracts were not always uniform. We have since further revised the method of preparation and introduced a number of additional procedures, which enable us to prepare extracts of high and uniform potency. In our most recent studies on fractionation of the extract containing the life-maintaining hormone, it appeared that the major part of the potency is contained in a single fraction. The extract thus obtained is of significantly greater physiological activity than our routine extracts. One of the side fractions consists mainly of the acid sodium salts of fatty acids, a fact which appears highly important with respect to their action as activators of the male sex hormones.

EXPERIMENTAL. *Assay method.* In most of our earlier work we used rats, cats, and the smaller laboratory animals. Recently we have utilized a number of dogs in order that comparison of our results might be made with those obtained by other workers.

Medium-sized male dogs weighing between 6 and 12 kilos were used as test animals. They were kept on a mixed diet, and no extra salt was allowed. Adrenalectomy was performed in two stages with an interval of 10 to 30 days. Subcutaneous injections of 5 cc. of the routine extract

[1] Grateful acknowledgment is made of aid received from the Rockefeller Foundation.

(daily) were started three days before the second operation, and rather large amounts (from 12–20 cc.) were thereafter given for about 7 days. By this time the animals had overcome the shock of operation and could be used for assaying different extract fractions. The smallest daily dose of extract which was sufficient to keep a dog in a normal condition, divided by the animal weight in kilos, was defined as one dog unit; i.e.,

$$\frac{\text{Maintenance dose/dog/day}}{\text{Weight of dog in kilos}} = 1 \text{ dog unit.}$$

In practice, the daily dosage found necessary was given in two injections, about one-third in the morning and the remainder in the afternoon. It is to be emphasized, however, that our dog unit differs from that used by Pfiffner, Swingle and Vars (1934), since we do not introduce the factor of additional salt given with the diet. It is stated by many workers that the provision of extra salt in the food allows a remarkable reduction of the dosage of cortico-adrenal extract which is necessary to maintain life (Reichstein, Laqueur and collaborators, 1936). Regular determinations of blood urea nitrogen were carried out, and a sudden rise was considered indicative of approaching insufficiency. In the evaluation of extract potency, however, the experiments were continued until characteristic clinical symptoms (weakness, vomiting, loss of weight, etc.) had become apparent. The animals were kept on a fixed dosage for six days; then the amount of extract was halved. After successive further reductions of the dosage, a time was eventually reached when symptoms of insufficiency appeared. In cases in which adrenal insufficiency was allowed to develop through reduction of the extract, it was easily possible to restore the dogs to normal within a few days by the administration of larger amounts of extract (about three times the minimum amount).

Preparation of the routine extract.[2] The cattle adrenal glands used were shipped to the laboratory in carbon dioxide snow. The whole glands were dissected from fat, ground finely in a meat grinder and placed in 95 per cent alcohol, in the proportion of 2 liters of alcohol to one kilo of glandular tissue. The mixture was frequently stirred (by motor stirrer) while heated for three days at 38 to 40°C. The supernatant fluid was decanted and the tissue residue reground and reëxtracted with 80 to 85 per cent (redistilled) alcohol. All the alcoholic solutions were then concentrated in vacuo to about one-sixteenth of the original volume, at a temperature not exceeding 40°. The non-homogeneous aqueous residue was shaken several times with benzene. When the benzene layer was almost colorless, the aqueous phase was saturated with sodium chloride and the benzene treat-

[2] The modifications were worked out with the assistance of Mrs. Elsa Ehrenstein.

ment repeated. The combined benzene extracts were concentrated in vacuo at a temperature below 40°. It was found advisable to secure about 100 cc. of a concentrated benzene extract from about 10 kilos of glands. The phospholipids were precipitated by the addition of about 500 cc. of pure acetone. After standing in the ice-box for about 30 minutes, the supernatant fluid was decanted. The precipitate was dissolved in a minimum amount of benzene (about 40–60 cc.) and the precipitation repeated by the addition of acetone (about 400–500 cc.). This procedure was carried out three more times. While the acetone was slowly added the mixture was vigorously shaken. Finally the combined benzene-acetone solutions were filtered and evaporated to dryness at a temperature below 40°. This method of precipitation secures a more uniform result and possibly also a better yield of hormone than the old method of extracting the *dry* residue with acetone. Subsequent procedures were carried out according to the method previously given by Britton and Silvette (1931).

To the final alcoholic solution obtained from 10 kilos of fresh glands 500 cc. of water were added. The mixture was brought to a volume of 250 cc. by distillation in vacuo at a temperature below 38°; thus 1 cc. refers to 40 grams of fresh glands. The aqueous solution was separated from the emulsified and suspended insoluble material by filtration through a Seitz-filter. This last manipulation required considerable time. The final aqueous solution, which was stored in the ice-box, was somewhat opalescent and showed a pale orange color. The solid content of these extracts was determined in several instances. On the average 25 cc. of the latter (= 1 kilo of fresh glands) contained 55 milligrams. Therefore 1 cc. of the extract contained 2.2 milligrams of solid material.

Physiological potency of the crude routine extract. The following animals were tested on samples derived from combined batches of extract, representing 200 kilos of fresh beef adrenal glands.

Dog 1. Adrenalectomized. Weight 7 kilos. Extract reduced to 5 cc. daily; no symptoms. Insufficiency six days after starting daily dose of 2.5 cc. (see assay method). Smallest necessary daily dose (estimated) 3.5 cc. Thus 1 cc. contains 2 dog units, and 1 mgm. solid extract about 0.9 d.u. Yield obtained from 10 kilos fresh glands (250 cc. of extract) approximately 500 d.u.

Dog 2. Adrenalectomized. Weight 9 kilos. Extract reduced to 7 cc. daily without symptoms. Insufficiency observed with 3.5 cc. extract daily on the seventh day. Smallest necessary daily dose 4.5 to 5 cc. Thus 1 cc. contains approximately 2 d.u. (corroborating findings on dog 1).

Dog 3. Adrenalectomized. Weight 7.5 kilos. Insufficiency appeared with dose of 2 cc. on the sixth day. Minimum daily dose 3 cc. Thus 1 cc. extract contains 2.5 d.u.

These findings obtained on three dogs indicate that the routine extract contains at least 2 dog units per cubic centimeter, possibly somewhat more.

The yield obtained from 10 kilos of fresh adrenal glands is thus at least 500 dog units.

Fractionation studies on the routine extract. The starting material for these studies was the final alcoholic solution which had passed through the permutit column. The equivalent of 150 kilos of fresh glands was evaporated to dryness in vacuo at a temperature not exceeding 40°. Weight of the sticky dry residue: 51 grams. The residue was treated with 5 liters of acetone (reagent) and allowed to stand in the ice-box over night. A brown powdery precipitate appeared. The supernatant fluid was siphoned off and the remainder filtered. Weight of the dry precipitate (A): 4.1 grams.

The acetone filtrate was brought to dryness at a temperature below 40°. To the residue were added 3 liters of benzene (reagent); the mixture was shaken and then kept cool over night. The solution was separated from the partly sticky and partly crumbly precipitate by filtering. Weight of precipitate (B): 3.3 grams.

The benzene solution was evaporated to dryness in vacuo and the residue treated with 3 liters of petroleum ether (B.p. 30–60°). At first there appeared to be a complete solution; the fluid suddenly became cloudy, however, and a very light brown powdery precipitate settled out. The whole was thoroughly mixed and then kept in the ice-box over night. The precipitate was decanted seven times with 600 cc. of pure petroleum ether, and afterwards brought to dryness. Weight of the precipitate (C): 4.4 grams.

The petroleum ether solution was likewise evaporated to dryness. The residue (D. Weight: about 40 grams) represented a partly sticky and partly crystalline brown mass which consisted mainly of cholesterol.

The precipitates A, B, and C were kept in alcoholic solution in the cold until examined for physiological potency.

Precipitate A, containing an acid salt of palmitic acid. When treated with 240 cc. of 95 per cent alcohol at room temperature for several days, more than one half of the precipitate went into solution. The insoluble material was a dark brown powder (1.4 gram). By evaporation of the alcoholic solution to dryness in vacuo a tan-colored powder (2.5 grams) was obtained which was treated with 220 cc. of water. The insoluble part appeared as a veil of microscopically fine silky needles. These were too delicate to filter and were separated by centrifuging in the ice-box; finally the crystals were freed from water on a clay plate. Yield: 0.78 gram of dry material. The substance was re-crystallized from 18 cc. of 95 per cent alcohol; thus 0.63 gram of rosette-grouped microscopically fine flat needles of constant M.P. 119–123° (slight sintering at about 75°) was obtained. Microanalysis (Dr. A. Schoeller, Berlin-Schmargendorf):

Found:	C 72.00%	H 11.81%	Na 4.38%
$C_{16}H_{32}O_2$:$C_{16}H_{31}O_2Na$ requires:	C 72.05%	H 11.85%	Na 4.31%

The free acid, obtained from this salt, was re-crystallized from 95 per cent alcohol; M.P. 56.5–57°. M.P. of purest palmitic acid "Kahlbaum": 60–62.5°. Mixed M.P. 58.5–60.5°. Micro-analysis (Chemical Laboratory, University of Virginia):

Found:	C 74.97%	H 12.54%
$C_{16}H_{32}O_2$ requires:	C 74.92%	H 12.59%
$C_{18}H_{36}O_2$ requires:	C 75.98%	H 12.77%

As a further check the isolated palmitic acid was identified through the phenacyl ester (Rather and Reid, 1919). It should be mentioned that the above treatment of precipitate A was carried out with several batches of cortico-adrenal extract. There appeared to be a constant yield of the acid sodium salt of palmitic acid.

Precipitate B. To date no further fractionation of this material has been carried out.

Precipitate C. This material had been dissolved in 100 cc. of alcohol. Eighty cubic centimeters of this solution (corresponding to 120 kilos of fresh glands) was evaporated to dryness in vacuo. Weight of the dry residue: 3.57 grams. The latter was treated thirty times with 100 cc. of benzine (reagent) at room temperature. Three liters of a yellow-brownish benzene solution was thus obtained, which contained the major part of the material (C2). Weight of undissolved brown residue (C1): 0.75 gram.

Two and one-half liters of the benzene solution C2 (containing 2.35 grams of solid material and representing 100 kilos of fresh glands) was treated in accordance with the chromatographic adsorption technique of Tswett, modified by Winterstein (1934). A mixture of 75 grams of anhydrous aluminum oxide (standardized according to Brockmann) and 3 grams of "fasertonerde" (Wislicenus)[3] was used as the adsorptive agent. A Jena fritted glass filter (no. 15aG3) served as the adsorption column.

Three main colored zones could be observed:

1. Upper zone (C2a), 2.5 cm. broad, brown, homogeneous.
2. Middle zone (C2b), 3.8 cm. broad, light reddish-brown, homogeneous.
3. Lower zone (C2c), 4.4 cm. broad, light olive-green; subdivided into five zones with different shades.

The filtrate was completely colorless. When brought to dryness no residue was obtained.

The zones were separated and treated while still wet. After extraction fifteen times with an appropriate amount (15–40 cc.) of methyl alcohol (reagent), the adsorptive agent appeared colorless, except in the case of zone C2a, in which a yellow color was tenaciously retained. The methyl alcohol solutions were brought to dryness; the sum of the dry residues

[3] Both chemicals were obtained from E. Merck, Darmstadt (Germany).

was 2.13 grams. (C2a, 0.721 gram; C2b, 0.790 gram; the fractions of C2c contained the rest.)

To the different residues were added small quantities of methyl alcohol. After standing in the ice-box for several days, a small quantity of an apparently amorphous, white precipitate was obtained from fraction C2b. Its melting point was 92 to 94°. The substance[4] is soluble in warm ethyl alcohol, ethyl acetate and dioxane. Micro-analysis (Dr. A. Schoeller, Berlin-Schmargendorf):

Found: C 74.03% H 12.19% N 2.30%

Physiological potency of the fractions described above.[5] *Precipitate B.* One cubic centimeter of the clear (light brown) aqueous solution (representing 40 grams of fresh adrenal glands) contained 0.9 mgm. of solid material.

Dog 1. Adrenalectomized. Weight 6 kilos. Twelve cubic centimeters of extract given for seven days and 5 cc. for further seven days. A sharp rise of blood urea then appeared, also insufficiency symptoms. Minimal daily dose (estimated) 7.5 cc. Thus 1 cc. represents 0.8 d.u., and 1 mgm. solid material contains 0.9 d.u. Referred to 10 kilos glands, the yield is approximately 200 d.u.

Precipitate C. As in the case of precipitate B, the substance was readily soluble in water. One cubic centimeter of aqueous solution (= 40 grams of fresh adrenal glands) contained 1.2 mgm. solid material. In a layer of several centimeters the clear solution showed a yellow color, and was distinctly fluorescent; the solution also tasted bitter.

Dog 1. Adrenalectomized. Weight 6.5 kilos. Dog maintained on this extract about 4 weeks. With a daily dose of 2.2 cc., no symptoms appeared. Insufficiency observed, after dog kept on dose of 1 cc. six days. Minimum daily amount of extract (estimated), 1.5 cc. Thus 1 cc. represents 4.3 d.u., and 1 mgm. solid material contains 3.6 d.u.

Dog 2. Adrenalectomized. Weight 8.5 kilos. Symptoms few days after dog put on daily dose of 2.2 cc. Minimum daily dose (estimated), 3.3 cc. 1 cc. represents 2.6 d.u.; 1 mgm. solid material contains 2.2 d.u.

According to data obtained from the two dogs above, the yield of physiological activity obtained from 10 kilos of fresh adrenal glands is 1075 and 650 d.u. respectively.

Fractions obtained from precipitate C. Fraction C1. One cubic centi-

[4] Melting point and analysis resemble those of a new estrogenic substance recently obtained by Andrew and Fenger (Science **84:** 18, 1936). Our substance was ineffective when injected into ovariectomized rats in daily doses as high as 10γ.

[5] In preliminary experiments which were carried out some time ago on cats, no evidence of the potency of precipitate "A" was obtained. They yielded evidence of some potency in the precipitate "B." By far the major part of the physiological activity was found in precipitate "C." No potency was found in the residue "D."

meter of the aqueous solution (equivalent to 40 grams of fresh adrenal glands) contained 0.25 mgm. of solid material. When injected daily with 5 cc. of this solution, a dog showed symptoms within two days. The potency of this fraction is thus negligible.

Fraction C2. Five hundred cubic centimeters of the benzene solution (equivalent to 20 kilos of fresh glands) was brought to dryness; weight of the residue: 0.50 gram. The latter was kept in a solution of 100 cc. of alcohol until tested for potency. The aqueous solution assayed showed a slightly yellow color, was somewhat fluorescent and tasted bitter. One cubic centimer of this fluid (equivalent to 40 grams of fresh adrenal glands) contained 1.0 mgm. of solid material.

Dog 1. Adrenalectomized. Weight 6 kilos. Assay made over three weeks; no symptoms on daily dose of 2.2 cc. When dosage was reduced to 1.1 cc., insufficiency appeared in 4 days. About 1.8 cc. might be considered the minimum daily dose. Thus 1 cc. (= 1.0 mgm. of solid material) contained 3.3 d.u. Yield of potency per 10 kilos of fresh adrenal glands, 660 d.u.

Adsorption zones obtained from fraction C2. Zone C2a. An aqueous solution (slightly yellow) of the dry residue was prepared, 100 cc. containing 100 mgm. of solid material.

Dog 4. Adrenalectomized. Weight 6 kilos. Symptoms apparent when daily dose reduced below 1.7 cc. (= minimum daily dose). Thus 1 mgm. solid material contains 3.5 d.u. Physiological activity appeared to be no higher than that of the starting material (C2).

Remaining zones. These have not yet been thoroughly assayed. It should be mentioned however, that the white precipitate, obtained from fraction C2b (M.P.: 92–94°) was physiologically inactive when assayed on three dogs.

Discussion. Higher yields of the cortico-adrenal hormone secured by the precipitation method described may be explicable on the basis of several considerations. The aqueous phase which is finally obtained before Seitz filtration in the modified Swingle-Pfiffner process we employ (or in the original author's method) is really an emulsion. Passage through the filter is a very slow process and loss of hormone by adsorption[6] and perhaps also destruction takes place. Moreover, much of the active material may be rendered ineffective by the presence of substances which are toxic to the organism. Numerous investigators have testified within the past few years to the effects of such toxic bodies in the ordinary Swingle-Pfiffner extracts.

[6] T. Reichstein (Helv. Chim. Acta **19:** 1109, 1936) recently stated that he likewise obtained evidence of remarkable potency of the material retained in the Seitz filter. Swingle and Pfiffner (This Journal **98:** 148, 1931) claim that "a small amount of activity remains in the water-insoluble fraction."

The fractionation procedures described herein apparently eliminate much of the deleterious material and give a higher potency yield even on the basis of fresh gland used. Per unit of solid substance, the refined extracts showed twice the potency of the original ("routine") extracts. In this connection, it may be emphasized that the highly active precipitates "B" and especially "C" may be very easily obtained. Furthermore, they are readily and completely soluble in water and filtration losses are thus avoided. Precipitate "A" which consists mainly of acid soaps is especially interesting. It was definitely shown that the crystalline substance of M.P. 119 to 123° is an acid salt, consisting of one molecule of palmitic acid and one molecule of sodium palmitate. One must not necessarily make the assumption that these salts are originally present in the adrenal glands; they may have been formed during the permutit treatment. On the other hand, however, the occurrence of such salts with a pH slightly below 7 in body tissues does not appear improbable. The presence of free fatty acids in certain body tissues was recently discussed by Laqueur (David, Dingemanse, Freud and Laqueur, 1935) and his collaborators (Pollak, Dingemanse and Freud, 1936). These acids were found to be present in extracts obtained from testes and shown to be activators of certain male sex hormones when injected simultaneously with the latter. Tschopp (Miescher, Wettstein and Tschopp, 1936a) succeeded in isolating palmitic acid from the acid fraction of testicular tissue. Palmitic acid has been proved to be a strong activator of male sex hormones (Miescher, Wettstein and Tschopp, 1936b; Deanesly and Parkes, 1936). According to the collaborators of Laqueur (Pollak, Dingemanse and Freud, 1936) activators even stronger than palmitic acid must be present in those acid fractions. Their chemical nature is thus far unknown and has been designated x-substance.

Recently we have obtained evidence on young castrated male rats (development of seminal vesicles and prostate gland) that the activating effect of the acid sodium salt of palmitic acid on testosterone is several times greater than that of free palmitic acid.

Thus we believe that in testes and in adrenal glands the free acids may be primarily present as acid sodium salts. When injected into body tissue, free palmitic acid is possibly converted into the acid sodium salt which may represent the real activator. Since Reichstein (1936) demonstrated the presence of male sex hormones in the adrenal cortex, the question of the presence of activators of such hormones in the same gland assumes considerable importance. We have obtained evidence of the presence of acid soaps other than that of palmitic acid, and are at present studying their possible action as activators of male sex hormones.

SUMMARY

A modified method for the preparation of cortico-adrenal extract for use in routine studies is described. By its use adrenalectomized dogs may be maintained alive indefinitely in apparently normal health.

By means of fractionation procedures more refined extracts (especially "C") showing much greater physiological activity have been obtained. Such relatively pure extracts contained up to twice the number of dog units per kilo of fresh gland tissue and only one-half the amount of solid material present in the routine extracts. The extracts "B" and "C" were moreover readily and completely soluble in water and thus filtration was avoided.

An acid sodium salt of palmitic acid, $C_{15}H_{31}COOH:C_{15}H_{31}COONa$, has been isolated from extracts derived from the adrenal cortex. Its considerable importance as an activator of male sex hormones is discussed. Evidence of the presence of acid soaps other than that of palmitic acid was obtained.

A fractionation procedure for cortico-adrenal extracts utilizing the chromatographic adsorption technique is described.

The composition and melting point of a new, yet amorphous carbon compound derived from the adrenal glands is described. It was ineffective on adrenalectomized animals and on ovariectomized rats when given in appropriate doses.

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CARBOHYDRATE MOBILIZATION

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With the increasing number of pathological conditions attributed to disturbances of the sugar level of the blood more attention is being paid to factors influencing carbohydrate mobilization. When glucose is absorbed, that which is not immediately utilized for heat and energy is stored as glycogen, for the most part, in the liver. This glycogen forms a readily available source of glucose for the restoration of depletion of the sugar in the circulating fluids.

The level of the sugar in the blood is maintained by a very complex mechanism which efficiently balances the intake and output from the blood stream. Some work has been done and is being done in these laboratories on the factors which deplete blood sugar by way of the insulin output (1, 2, 3). In studying the factors which restore the lowered blood sugar, most attention has been paid to the rôle of the adrenal glands. The influence of the adrenal glands has been shown to be on the liver, promoting glycogenolysis. Severe damage of the liver by such agents as chloroform, phosphorus, carbon tetrachloride and arsenic, interferes with this glycogenolysis (4, 5, 6) and, as a consequence, the blood sugar is lowered. However, little attention has been paid to disturbances of this function where no hepatic pathological change can be demonstrated.

Most investigators who have worked on the influence of drugs on carbohydrate metabolism have assumed that if the blood sugar level does not change at the height of the drug action, there has been no disturbance of carbohydrate function. Recent work with nembutal (7) has shown that though there is no change in the mean blood sugar level during the anesthesia there is a fall during recovery. A similar hypoglycemia has been found after the administration of pentothal (8), a closely related drug. These barbiturates produce an anesthesia of relatively short duration. Since they are detoxified in the liver (9), it was thought worth while to determine their effects on the glycogenolytic function of the liver.

The percentage increase in the sugar level following the administration of epinephrine is proportional to the dosage (10, 11), the greatest change taking place during the first half-hour after the injection. If the barbitu-

rates in question interfere with the glycogenolytic power of the liver, it might be expected that they would interfere with the effect of epinephrine. The work presented in this paper is a study of the effect of nembutal upon the epinephrine rise in the blood sugar level.

Normal-fed rabbits were given nembutal intraperitoneally, in the dosage of 40 mgm. per kilogram of animal. The anesthetized animals show signs of recovery in about $1\frac{1}{2}$ hours and complete recovery in about $2\frac{1}{2}$ hours. Epinephrine hydrochloride was given subcutaneously, 0.25 mgm. per kilo. The effect of epinephrine on the blood sugar level when injected at various stages of anesthesia were determined. Blood samples were taken 30 minutes and 60 minutes after the injection. The sugar was determined by the Somogyi micro method (12). Figure 1 shows very definitely that at the time of recovery from nembutal there is a profound suppression of

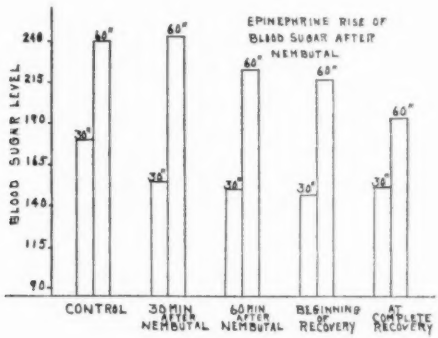

Fig. 1

glycogenolysis as evidenced by the diminished epinephrine effect. If epinephrine is injected 30 minutes after the administration of the nembutal there is already evident a considerable depression of the hyperglycemia. In the series taken at the beginning of recovery, 15 per cent showed slight or no epinephrine hyperglycemia, 50 per cent showed little or no change at the half-hour only and 35 per cent gave the usual normal rise. In the series injected with epinephrine at the time of complete recovery, 21 per cent showed little or no rise in the sugar at the end of $\frac{1}{2}$ or 1 hour, 30 per cent showed little or no change at the half-hour but an elevation at the hour and 49 per cent gave the normal hyperglycemia. This variation in the response of the animals to epinephrine is interpreted as being due, in the main, to the variations in the response of the animals to the nembutal. All animals were tested for the epinephrine response regardless of the degree of anesthesia induced by the nembutal. Those individuals which show

little reaction to the nembutal probably detoxify it more rapidly than those showing profound and prolonged depression.

There may be various explanations for the altered glycogenolytic activity of the liver. It seems reasonable to suppose that since the nembutal is withdrawn from the blood and detoxified in the liver, the hepatic cells may be depressed and their glycogenolytic function impaired.

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DEFICIENCIES IN THE RIGHTING REFLEXES OF CATS FOLLOWING BILATERAL CORTICAL LESIONS

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In an earlier communication (Barris, 1937) it was briefly mentioned that in cats from which the rostral portions of the neocortex had been removed, there were observed deficiencies in certain of the righting reactions. It is the purpose of the present report to consider more fully the abnormalities in the righting reflexes as observed in such animals.

The many studies of Magnus and his co-workers which Magnus (1924) has summarized have provided ample evidence to show that a normal animal maintains an upright posture and equilibrium by virtue of what Magnus termed the "righting reflexes" (Stellreflexes). These reflexes have been shown to be dependent upon the stimulation of receptors within the labyrinths, retinae, muscles of the body and neck, and skin of the body surface. They have been designated as 1, labyrinthine righting reflexes; 2, optic righting reflexes; 3, body righting reflexes, and 4, cervical righting reflexes.

Although Dusser de Barenne and Magnus (1920) were of the opinion that a decorticate or a "thalamus" cat was as capable of carrying out the various righting reflexes as a normal cat, and although Magnus (1924, 1925) believed that all of the righting reflexes with the exception of the optic righting reflexes were fully developed in "midbrain" and "thalamic" animals, the observations herein to be reported indicate that in cats the righting reflexes may not be fully developed following ablation of the rostral parts of the neocortex. In such animals it has been noted that there are deficiencies in the following righting reflexes: 1, labyrinthine; 2, body on the body, and 3, body on the head.

Observations have been made on both acute and chronic cats lacking the rostral portion of the neocortex. Eight cats with bilateral ablations were observed over periods ranging from one to two weeks, and three animals over periods ranging from eight to fifteen weeks.

METHODS. That portion of the neocortex lying rostral of a point two to three millimeters behind the medial tip of the ansate sulcus was removed bilaterally in a one-stage operation in all of the eleven cats observed. The

[1] Aided by a grant from the National Research Council.

TABLE 1

Showing degree of impairment of certain of the righting reflexes of cats following cortical injury

CAT	DAYS	FALLING IN AIR WITHOUT BLINDFOLD			FALLING IN AIR WITH BLINDFOLD			ON SUPPORTING SURFACE			
								Without blindfold		With blindfold	
		Back	R.S.	L.S.	Back	R.S.	L.S.	B.B.	B.H.	B.B.	B.H.
1	2	−	−	−	−	−	−	−*	N	−*	−
	3	−	−	−	−	−	−	−*	N	−*	−
	7	−	++	++	+	+	+	−*	N	−*	−
	10	++	++	++	−	+	+	+*	N	−*	I
	59	++	++	++	−	++	++	+*	I	−*	I
	75	++	++	++	−	+	+	+*	I	−*	−
	107	−	++	++	−	+	+	+*	I	−*	−
2	1	−	++	++	−	−	−	−		−*	−
	3	++	+	+	+	−	−	−*	N	−*	−
	6	−	++	++	−	+	+	−*	N	−*	−
	55	++	++	++	−	+	+	+	N	−*	−
	73	−	+	+	−	+	+	+	I	+*	I
	105	−	++	++	−	+	+	+*	N	+*	−
3	1	−	−	−	−	−	−	−	−		
	2	++	−	−	+	−	−	−	I		
	6	++	+	+	+	+	+	+*	I	+*	−
	9	−	++	++	−	+	+	−*	N	−*	−
	58	−	++	++	−	+	+	+	N	+*	−
4	1	−	−	−	−	−	−	+*	I	−*	−
	3	−	+	+	−	−	−	−*	N	−*	I
	7	+	++	++	−	+	+	+*	N	+*	−
	9	++	++	++	+	+	+	+*	N	−*	I
	12	+	++	+	+	+	+	+*	I	+*	−
	17	−	++	++	−	+	+	−*	I	−*	I
5	1	−	−	−	−	−	−				
	6	+	++	++	+	+	+	−*	I	−*	−
	9	+	++	++	+	+	+	−*	I	−*	I
	12	+	++	++	−	+	+	−*	N	−*	−
6	1	−	++	++	−	+	+	−*	N	−*	I
	2	+	++	++	−	+	+	−*	N	−*	I
	6	++	++	++	+	+	+	+*	N	−*	I
	9	+	++	++	+	+	++	−*	N	−*	I
7	2	−	−	−	−	−	−	+	I	−	−
	6	++	++	++	+	+	+	+*	I	−*	−
	9	++	++	++	+	+	+	+*	I	+*	−
8	1	−	+	+	−	−	−				
	3	−	+	+	−	+	+	+*	N	+*	I
	8	−	++	++	−	+	+	+*	N	−*	N
9	2	−	++	++	−	+	++	+	N	+*	−
	5	+	++	++	−	+	+	+*	N	+*	−
	8	+	++	++	+	+	+	+*	N	+*	I
10	2	−	−	−	−	−	−	−*	N	−	I
	3	−	−	−	−	−	−	−*	I	−*	−
	7	++	++	++	+	+	+	+*	N	+*	I
11	1	+	++	++	−	+	+	−*	I	−*	−
	2	+	++	+	−	+	+	+*	N	+*	−
	7	+	+	+	+	+	+	+*	N	+*	I

Abbreviations and symbols: R.S. From right side. L.S. From left side. B.B. Body on body. B.H. Body on head. − = reflex absent. + = delayed and incomplete. ++ = delayed and complete. N = normal. I = incomplete. * = tail pinched. Days = Days after operation.

operative procedures necessary for such ablation have been described in an earlier report (Barris, 1937). Twenty-four to forty-eight hours after operation, observations were made on labyrinthine, body on the body, and body on the head righting reflexes. Observations were repeated and recorded at frequent intervals thereafter (table 1).

OBSERVATIONS. *Labyrinthine righting reflexes.* In keeping with the considerations of de Kleijn and Magnus (1920), labyrinthine righting reflexes in the animals herein under consideration were tested with the animals blindfolded. If, under these circumstances, these animals were dropped into the air from a position on their backs or sides and from a height of about four feet, they either did not right themselves at all or the reaction was very delayed and often incomplete (table 1). When the animals were held in the air with the body in the side position, the head did not as a rule right itself, but remained in the position described by de Kleijn and Magnus (1920) for blindfolded labyrinthectomized cats (fig. 1). When suspended in the air in the back position, the head would gradually drop downward, and finally come to rest without righting (fig. 2).

If these animals were held by the pelvis and suspended in the air so that the bodies hung downward, the head hung downward and there was no evidence of any righting of the head (fig. 3).

The various "labyrinthine" righting reactions were also tested in these animals when not blindfolded. Under these circumstances, it was noted that if these animals were dropped into the air from a position on their backs or sides, they either did not right themselves at all, or the reaction was greatly delayed and often incomplete (table 1). This righting reflex appeared to be a trifle less deficient when the animals were not blindfolded than when blindfolded. When held suspended in the air so that the bodies of the animals were in the side, back, or upright position, the head usually assumed a normal posture (figs. 4 and 5). If these animals were held by the pelvis and suspended in the air so that the bodies hung downward, the head turned either to the right or to the left and partially righted itself (fig. 6).

Body on the body. If an animal was placed upon its side, and the head held firmly on the supporting surface, very little effort was made to right the body. Even with the application of painful stimuli, such as vigorous pinching of the tail, this reaction was either absent or greatly delayed and incomplete. Thus, the animals after painful stimulation might thresh their limbs about vigorously, and yet not succeed in elevating the body to an upright position. In some animals, after a number of abortive efforts, the hind part of the body might be righted, but it was only rarely that an animal succeeded in righting the entire body. When blindfolded, these animals would usually lie motionless upon their sides. It was always necessary to apply vigorous stimulation of a painful character, such as

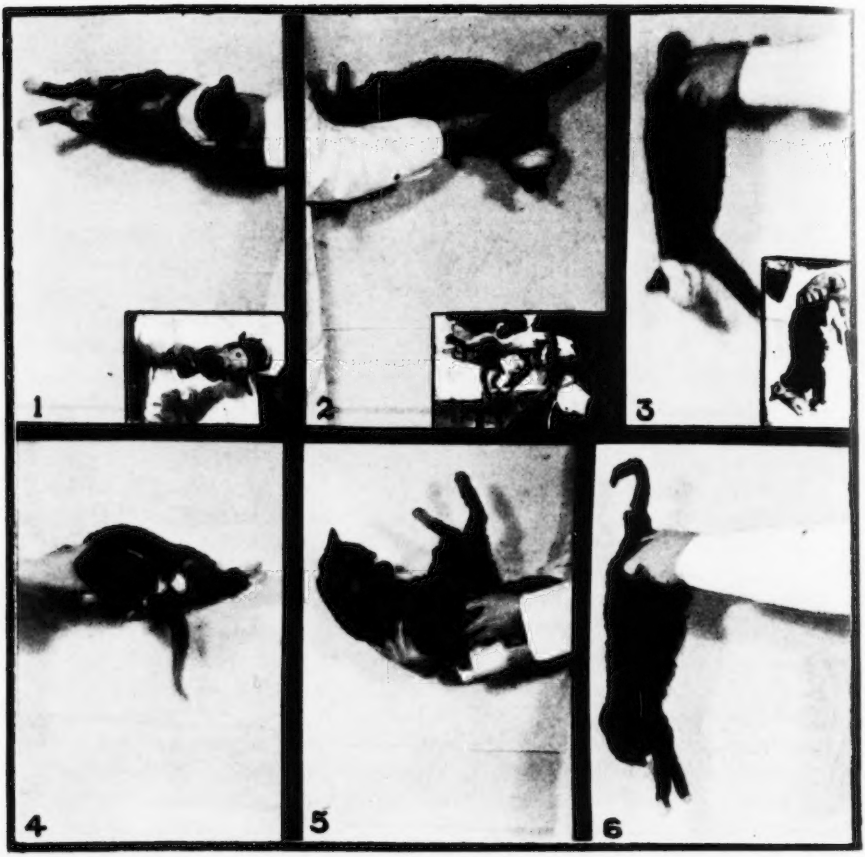

Fig. 1. Cat 1—Showing failure to bring head into normal position when held from side and blindfolded (deficiency of labyrinthine righting reflex).

Fig. 2. Cat 1—Showing failure to bring head into normal position when held on back and blindfolded (deficiency of labyrinthine righting reflex). In this photograph it might appear as if the blindfold was so applied that the head was held against the forepart of the neck, and thus prevented from reacting without restraint. However this may appear from this photograph, the head as a rule hung downwards in a position comparable to that shown in the lower right hand photograph of figure 2.

Fig. 3. Cat 1—Showing failure to bring head into normal position when suspended by pelvis and blindfolded (deficiency of labyrinthine righting reflex).

Fig. 4. Cat 1—Showing head in normal position when held on side without blindfold.

Fig. 5. Cat 1—Showing head in normal position when held on back without blindfold.

Fig. 6. Cat 1—Showing head in normal position when suspended by pelvis without blindfold.

Photographs appearing in lower right hand corner of figures 1, 2, and 3 illustrate the positions taken by the head in blindfolded-labyrinthectomized animals (taken from Pflüger's Arch. **180:** 291, 1920, A. de Kleijn and R. Magnus).

pinching the tail, to induce them to move the limbs. When the animals were blindfolded, the reflex was somewhat more deficient than without the blindfold (table 1).

Body on the head. If these animals were placed upon their sides, the body being held firmly against the supporting surface, the head as a rule righted itself as in a normal animal. Occasionally, however, the head was only partially righted, so that the head rested with the jaws against the surface of the table. A great deficiency in this righting reflex was observed when these animals were blindfolded. Under these circumstances, the head either did not right itself at all or righted itself only partially (table 1).

Maintenance of equilibrium in the standing posture. These animals were able to get to a standing position quite normally after they had been placed upon their side or back. If, when in a standing posture, they were forcibly pushed to one or the other side, they managed to preserve their equilibrium quite well. If, however, they were blindfolded, there at once became apparent certain equilibratory deficiencies, and they showed a great disinclination to walk. Under these circumstances, they would often assume a semi-crouching position or they would sit on their hind limbs and elevate the fore part of the body. This elevation of the fore part of the body was usually accompanied by excursive movements of the head so that it swayed from side to side. If these animals were then forcibly displaced sidewards or backwards, they would fall in the direction toward which they were forced, landing either on their side or back. If the tail was pinched, and an effort to walk was made, they appeared quite disoriented and ataxic and would frequently fall to one or the other side.

Discussion. From these experiments it is evident that all of the righting reflexes are not fully developed in cats following ablation of the rostral part of the neocortex. From the work of Magnus (1922, 1924, 1925) it has been generally accepted that all righting reflexes, with the exception of the optic righting reflexes, may be carried out as long as the midbrain is intact in an otherwise normal animal. It is difficult to harmonize this point of view with the observations in the present experiments. Admittedly, in this study, cats have been observed in which there had been only a partial cortical ablation. It may not then be proper to compare such animals with animals from which the entire cortex had been removed. It will be recalled that Dusser de Barenne and Magnus (1920) studied the righting reactions of two cats, one animal without the neocortex and the other animal lacking all of the cerebral cortex. A critical reading of the protocols presented by these workers will not allow one to conclude that in decorticate animals all righting reflexes, with the exception of the optic righting reflexes, are demonstrable. When one of their cats was dropped into the air from a position on its back, it showed a very tardy righting re-

action. The other of their cats did not right itself at all under similar circumstances, even as long as seventy days after operation.

That this righting reaction is very deficient in decorticate cats has also been shown by Bard and Rioch (1937). Reporting their observations on four cats without a neocortex, these investigators said: "Although our animals righted themselves very well when in contact with a supporting surface, they showed a marked deficiency in turning over in the air when dropped back down. The normal cat allowed to fall in this manner turns at once and lands on its feet even when the fall is one of only a few inches. The four subjects of our study accomplished this maneuver only when dropped from a height of several feet. Occasionally they did not turn in the course of a fall as much as four feet."

The animals in the present experiments brought their heads into a normal position when they were suspended in the air on their side or back, or when they were supported by the pelvis with the body hanging downward. These righting reflexes acting on the head were not demonstrable, however, when the animals were blindfolded. In this respect, they resembled the blindfolded labyrinthectomized cats of de Kleijn and Magnus (1920). It appears, therefore, that in these animals the labyrinthine righting reflexes are not fully developed.

The body on the body righting reflexes also appeared to be deficient in the animals herein under consideration. Unless painful stimuli, such as pinching the tail, were applied, these animals, with or without blindfold, did not execute this reflex righting of the body in the manner of a normal animal. It is curious that the two "gehirnlosen" cats of Dusser de Barenne and Magnus (1920) also required, as a rule, the application of painful stimulation of the tail in executing the body on the body reflexes. And even under these circumstances, the reflex was, according to their protocols, not always demonstrable. The hypothesis of Magnus (1924, 1925) is that asymetric stimulation of the body surface is responsible for the body on the body reflex. This cannot be harmonized with the necessity for application of painful stimuli in demonstrating the body on the body reflexes in "gehirnlosen" cats.

Until we know more about the very complex functions of the cerebral cortex, it will not be possible to account for righting reflex deficiencies in consequence of cortical injury.

SUMMARY

1. Observations on certain of the righting reflexes have been made on acute and chronic cats from which have been removed the rostral portions of the neocortex.

2. It has been noted that there are deficiencies in labyrinthine, body on the body, and body on the head righting reflexes.

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THE EFFECT OF OESTRONE ON THE SECRETION OF THE GONADOTROPIC COMPLEX AS EVIDENCED IN PARABIOTIC RATS[1]

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It has been adequately demonstrated that oestrin inhibits the normal development of the male and female gonad, and reduces the gonad-stimulating capacity of the anterior pituitary of castrated male and female rats (Haberlandt, 1927; Leonard, Meyer and Hisaw, 1931; Moore and Price, 1930; Meyer, Leonard, Hisaw and Martin, 1932; and others).

Kallas (1930) showed that the hypertrophy of the ovaries of a female rat in parabiosis with a castrated female could be prevented by the administration of oestrin to the castrated partner. Similar injections were without effect upon the hypertrophy resulting from parabiosis with a castrated male. On the basis of these findings Kallas believed that the limitation of gonadotropic hormone secretion by the hypophysis by oestrin obtains only in the female. Martins and Rocha (1931) were able to prevent ovarian stimulation of female rats in parabiosis with castrated male rats by subcutaneous implantation of fresh, macerated rat testicle into the castrated males, but not in females joined to castrated females which had also received testis tissue. These investigators also concluded that they had demonstrated a sex-specific suppression of hypophyseal gonadotropic secretion.

It thus became of interest to determine quantitatively the effect of oestrone on the amount of gonadotropic secretion of castrated male and female rats in parabiosis with normal females.

MATERIALS AND METHODS. This study includes observations made upon a total of 141 pairs, including 55 control pairs and 86 experimental pairs treated with varying amounts of oestrone. In order to test the reliability of our methods animals from two different colonies were used. The operative procedures used in uniting the animals were those described by Bunster and Meyer (1933) except that metal skin clips were used for closing the skin wounds instead of silk sutures. Their procedure allows for exten-

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sive vascular anastomosis in the muscles of the abdominal and thoracic region and makes for adequate mechanical stabilization of the union.

The operation was conducted under sterile conditions and under ether anesthesia. By careful post-operative care the mortality was reduced to 5 per cent.

Littermates were always used and as seen in table 1, 75 of the pairs from the "Z" colony and 42 pairs from the "S" colony were 30 to 33 days of age at the time they were put in parabiosis and one partner castrated. Autopsy

TABLE 1

Ovarian weights of female rats in parabiosis with castrated male or females given varying quantities of oestrone

COLONY	NUMBER OF PAIRS	SEX OF PAIRS	AMOUNT OESTRONE PER DAY	MEAN OVARIAN WEIGHT ± S.E.
			gamma	*mgm.*
Z^1	7	♀ ♀	0 05	24 0 ± 4 8
Z^1	6	♀ ♀	0 20	13 7 ± 1.14
Z^1	3	♀ ♀	1 00	13 4 ± 1 13
Z^1	2	♀ ♀	5 00	
Z^1	11	♀ ♀	None (control)	71 0 ± 12 4
Z^1	8	♂ ♀	0 05	42 5 ± 12 1
Z^1	11	♂ ♀	0 20	18 4 ± 1 7
Z^1	7	♂ ♀	1 00	15 7 ± 1 0
Z^1	4	♂ ♀	5 00	
Z^1	16	♂ ♀	None (control)	72 8 ± 11 4
Z^2	2	♀ ♀	5 00	
Z^2	3	♂ ♀	1 00	12 15 ± 1 0
Z^2	8	♂ ♀	5 00	
Z^2	5	♀ ♀	None (control)	81 8 ± 18 3
Z^2	6	♂ ♀	None (control)	85 8 ± 18 3
S^1	8	♀ ♀	0 20	14 0 ± 0 62
S^1	9	♀ ♀	None (control)	90 3 ± 10 3
S^1	10	♂ ♀	0 20	51 8 ± 11 7
S^1	7	♂ ♀	1 00	13 57 ± 0 67
S^1	8	♂ ♀	None (control)	81 2 ± 13 2

was done on the morning of the eleventh day after parabiosis. The remaining 24 pairs from the "Z" colony were the same age at the time of parabiosis but were not castrated until seven days after the operation; these were also killed and examined on the morning of the eleventh day after castration. At the time of autopsy the ovaries of the female parabionts were dissected free of extraneous tissue and rapidly weighed to the nearest milligram.

The oestrone used in the experiments was crystalline (m.p. 252°C.) and

regardless of the amount was always administered subcutaneously in 0.01 cc. corn oil per day.[3] Observations were made upon the gross qualitative condition of the ovaries. In addition, the size of the uterus and the time of vaginal opening was noted. Injections were always begun within a few hours after the removal of the gonads. The daily quantity of oestrone administered varied from 0.05 to 5 gamma.

The quantity of oestrone administered per day, the mean ovarian weight of each series, etc. are to be found in table 1. The data are unselected and represent our findings in all pairs surviving the experimental procedures in good general health. It was necessary to discard the data from four pairs which developed such extensive infection as to make it doubtful that their responses were normal.

Results and discussion. The close agreement between the mean ovarian weights obtained in the control series of castrated male-female and castrated female-female pairs causes us to believe that the parabiotic method yields sufficiently constant data to establish its usefulness for a quantitative study of hypophyseal gonadotropic secretion. Although the data are in good agreement, it is important to report that in 15 per cent of the control pairs there was no detectable ovarian stimulation, and that these pairs were indistinguishable from others in rate of healing and absence of infection. These findings make it essential that the ovarian response of adequately large groups of control and experimental pairs be statistically compared for the interpretation of quantitative data obtained by the parabiotic method. Accordingly, we have presented in table 1 the mean ovarian weight and the standard error of each control and experimental series. By the use of Fischer's formula for small numbers (Davenport and Ekas, 1936) we have determined whether the differences between the mean ovarian weight of certain of the series are statistically significant. (If the value of P was less than 0.05 the difference was considered statistically significant.) It should be emphasized that the rats were from two different colonies and under different laboratory conditions until they were 21 days of age and that the "Z^2" series were 47 to 50 days of age at the time they were killed and those of the "Z^1" and "S^1" series, 40 to 43 days old. Although these differences are present, there is no statistically significant difference in the mean ovarian weights of the six control groups.

It is clear from the data presented in table 1 that 0.20 gamma of oestrone per day will prevent any ovarian hypertrophy in pairs from the "Z" colony regardless of the sex of the castrated parabiont. When the daily dose of oestrone is reduced to 0.05 gamma per day the ovarian hypertrophy of the castrated male-female pairs of the "Z" colony is not totally prevented (42.5 ± 12.1 mgm.). The difference between the mean ovarian weight of

[3] We are indebted to Dr. G. F. Cartland of the Upjohn Co. for the oestrone used in these studies.

the castrated male-female pairs of the "Z" colony given 0.05 daily and that of the castrated female-females given the same amount per day (24.0 ± 4.8 mgm.) is not statistically significant but the latter differ significantly from the untreated control castrated female-female pairs (71 ± 12.4 mgm.). A statistically significant sex difference in this regard is noted in the pairs of the "S" colony injected with 0.20 gamma of oestrone per day. This quantity prevented any ovarian hypertrophy in the castrated female-female pairs and decreased it in the castrated male-female parabionts (14.0 ± 6.2 mgm. and 51.8 mgm. ± 11.7 respectively). The difference in the degree of ovarian hypertrophy of castrated male-female and castrated female-female pairs injected with the same quantity of oestrone presumably reflects a greater potency of the castrate male gland. It is suggested that the sex difference in response to oestrone which Kallas (1930) has reported rests upon this quantitative rather than upon a qualitative basis.

When the quantity of oestrone was increased to 1.0 gamma or more per day the prevention of ovarian hypertrophy was always complete regardless of the sex of the castrated partner. Thus, as the quantity of oestrone is increased the degree of ovarian hypertrophy resulting from castration of either the male or female rat is further decreased to a point where no hypertrophy is obtained.

The ovaries of the control and experimental series, when stimulated, showed no qualitative difference in response. The larger ovaries were always composed of both corpora lutea and follicles, the corpora predominating.

Martins and Rocha (1931) have emphasized the apparent inhibitory effect of the prepubertal gonad upon the hypophysis. The minute quantities of oestrin capable of suppressing the gonadotropic complex of the anterior pituitary are presumably proportional to the normal output of the immature ovary. It is accordingly difficult to visualize the normal approach of puberty except in terms of increasing gonadotropic potency of the pituitary gland without proportionally greater oestrone secretion by the prepubertal ovary.

The findings here presented are readily correlated with the morphological data offered by Nelson (1933, 1935) and Hohlweg and Dohrn (1931). These workers noted that oestrin was able to prevent castration changes in the hypophyses of both male and female rats, the former requiring several times as much oestrin as the latter. Our data similarly indicate a difference between the minimal inhibitory dose in castrated male-female pairs and that required for castrated female-females.

Nelson (1933) states that after the first ten days following castration the hypophysis of the rat shows only indecisive morphological changes, a longer period being required for the development of the typical castrate

picture. The methods here employed indicate that physiologically the hypersecretion of gonadotropic complex is evident before morphological changes of a definite type are present in the anterior pituitary.

The parabiotic method presents distinct advantages as a quantitative method for the study of the rate of hypophyseal gonadotropic secretion under experimental conditions. In the first place, it is probable that the amount of ovarian hypertrophy obtained is a measure of the quantity of the gonadotropic complex actually secreted by the pituitary into the blood stream, whereas the implant method of assay indicates the residual amount of hormone momentarily contained in the glandular tissue. In addition, the parabiotic method permits one to study the activity of the hypophysis in its normal environment rather than in an unusual graft site.

The distinct limitations of the method with respect to quantitative variability can be offset only by sufficiently large numbers of data with adequate statistical analysis.

SUMMARY AND CONCLUSIONS

The degree of ovarian hypertrophy which occurs when an immature female rat is put into parabiosis with a castrated male or female littermate is employed as a quantitative measure of the level of hypophyseal gonadotropic secretion.

Observations are presented on 141 pairs, including 55 untreated control pairs and 86 pairs treated with varying doses of crystalline oestrone in oil.

The data indicate that:

1. The parabiotic technique is adaptable to quantitative studies provided a substantial number of data are obtained.

2. The administration of small doses of oestrone to the castrate partner completely inhibits the ovarian hypertrophy usually observed in the normal partner, and the degree of ovarian response increases as the dose of oestrone is reduced.

3. Larger doses of oestrone are required for inhibition in castrate male-female than in castrate female-female pairs.

Discussion is presented regarding the bearing of these findings upon the reciprocal hypophyseal-gonadal relationship.

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THE EFFECT OF HYPOPHYSECTOMY ON ARTERIAL BLOOD PRESSURE OF DOGS WITH EXPERIMENTAL HYPERTENSION[1]

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Experimental hypertension can be produced in animals by applying clamps to the renal arteries. This important observation of Goldblatt, Lynch, Hanzal and Summerville (1) has made it possible to study with reasonable accuracy the effects of various surgical procedures on the level of arterial pressure.

Numerous investigators, notably Cushing (2), believe that the hypophysis is concerned in the genesis of hypertension of the essential variety, and of that associated with pituitary basophilism and eclampsia. Manifestly, it is impossible in patients to ascertain the participation of the pituitary gland by its removal. But in dogs with experimental hypertension this can be done. We have therefore sought to find whether the hypophysis is concerned in the genesis of hypertension resulting from constriction of the renal artery.

METHOD. Both male and female dogs of adult ages were employed. Arterial blood pressure was measured daily by the same investigator in a warm room (80°F.). The method of Van Leersum (3) was used, which consists in ascertaining the pressure at which beats come through a loop of carotid artery compressed by a rubber pressure cuff. In a number of instances, blood pressures were also measured by auscultation over an artery of the hind leg. The artery was compressed by a 5 cm. wide rubber cuff wrapped around the leg just above the ankle joint. By this method both systolic and diastolic pressures could be determined. The two methods agree within close limits in our hands.

Blood pressure measurements were made until we felt certain that the normal variations were known. Under pento-barbital anesthesia, an adjustable silver clamp was applied to the right renal artery according to the method of Goldblatt, Lynch, Hanzal and Summerville (1). If the blood pressure rose markedly and remained steadily elevated for 30 days

[1] A preliminary report of this investigation was made in Proc. Soc. Exper. Biol. and Med. **34**: 260, 1936.

or more, the hypophysis was removed. But if the pressure tended to fall, a clamp was applied to the left renal artery. In some instances, it was necessary to tighten the clamp, but in most, a steady, elevated, systolic and diastolic pressure was obtained. The hypophysis was removed under visual guidance by the subtemporal approach according to the method of Sweet (4). This consists essentially of exposing the gland through a subtemporal approach, enclosing the gland within a rounded cup followed by severance of the stalk. The hypophysis was removed intact, consequently it was not necessary to kill the animal to be certain that the operation had been complete. After operation, the animals were fed large amounts of carbohydrate, or glucose was given by stomach tube if their appetite failed. Usually the animals were up and had good appetites a day or two after operation.

The order in which the various operations were performed varied in different animals. In some, the clamps were applied and then the hypophysis removed, in others the order was reversed. In still others, after clamping and hypophysectomy, the clamps were tightened in an effort to reëstablish hypertension. In all, 17 dogs have been studied, some for two years.

The effect of thyroid, theelin and antuitrin S has also been studied on the arterial pressure of the dogs after clamping and hypophysectomy. The dosage of thyroid varied from 0.6 to 1.4 gram a day. Theelin and antuitrin S were administered intramuscularly in doses of 1 cc. a day.

Results. Pressure of the clamp on the renal artery produced a marked rise in both systolic and diastolic pressure in all of the dogs, as Goldblatt, Lynch, Hanzal and Summerville (1) have shown. The elevated pressure was maintained for many weeks or months before hypophysectomy was performed. The latter operation was followed invariably by a marked reduction in arterial blood pressure. The rapidity of fall varied among the animals, but usually 10 to 20 days were required for the maximum fall to occur in both systolic and diastolic pressure. The pressure was maintained in some dogs at a level usually about 20 to 30 mm. Hg above the level normal for the individual dog (fig. 1), but in others it sank well below the normal level (fig. 2). For example, the systolic pressure fell as low as 76 mm. Hg and remained low for several days, yet the animal appeared to be in excellent health. In order to be certain that the operation alone, without removal of the hypophysis, did not affect the arterial pressure, a control operation was performed on one dog and 12 days later the gland was removed by approaching it from the opposite side. No fall in pressure resulted when the gland was not removed, but removal caused the usual reduction in pressure.

Four normal dogs were subjected to hypophysectomy to ascertain the effect on the normal blood pressure. Two of them exhibited a fall from an

average level of 130 mm. Hg to a level of 110 to 120 mm. (confirming the results of Braun-Menendez, 5), while the pressure in the other two did not appear significantly altered.

Among the 17 dogs studied, marked variability was observed in response of the arterial pressure to hypophysectomy and subsequent increase of

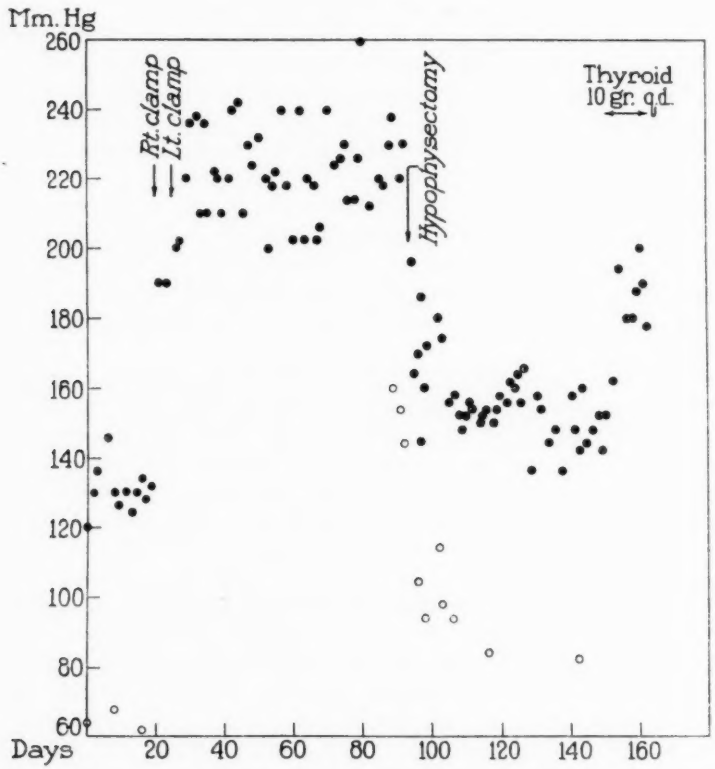

Fig. 1. Effect of hypophysectomy on systolic (solid dots) and diastolic pressure (circles) of dog with hypertension.

renal arterial constriction by the clamp. The arterial pressure fell to levels well below normal in 5 of the dogs and remained so, except for minor interludes, for periods up to 2 years. In these animals it has been impossible to produce more than a transient rise in pressure by further constricting the renal artery. Clinically, they were characterized by the facts that they all increased markedly in weight (one rose from 13.5 kgm. to 30.0 kgm.), they were docile, quiet and somewhat sluggish, their appetites were excel-

lent, their coats were soft, luxuriant and tended to shed and be replaced frequently, and they all had polyuria. The basal metabolic rate was

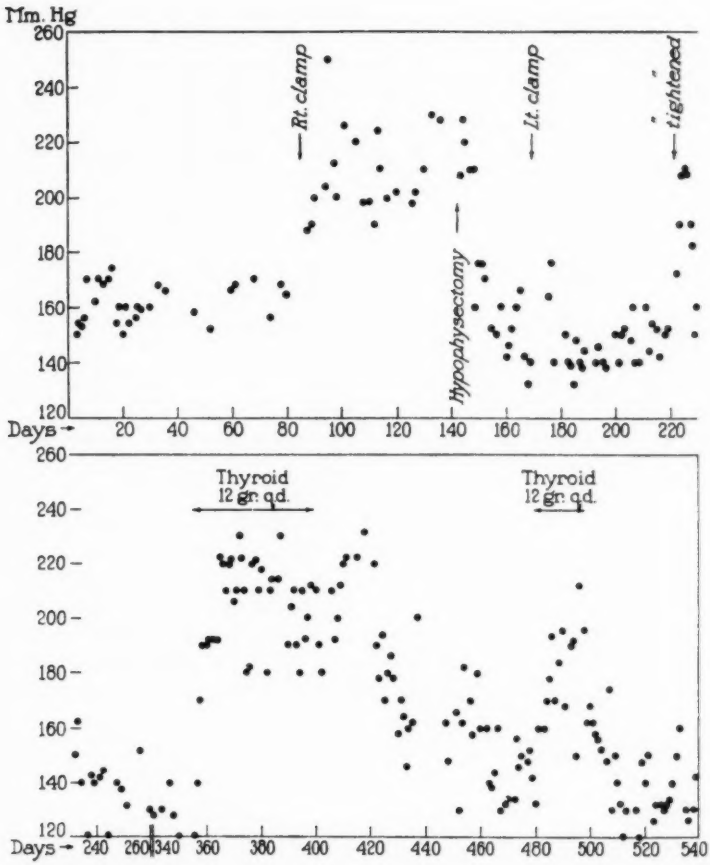

Fig. 2. Effect of hypophysectomy and thyroid feeding on arterial pressure of a dog. A portion of the chart has been deleted (between 260 and 340 days) which represented daily blood pressure measurements varying from 98 to 140 mm. Hg for a period of 80 days.

measured in one animal of this group and was found reduced (13 per cent of normal).[2]

Contrasting with this group of animals were those in which the fall in

[2] We wish to express our appreciation to Dr. D. Holman for making these measurements.

blood pressure seldom was less than the normal level for the dog and usually was 20 mm. or more above it. Increase in the constriction of the renal artery subsequent to the hypophysectomy usually resulted in a definite

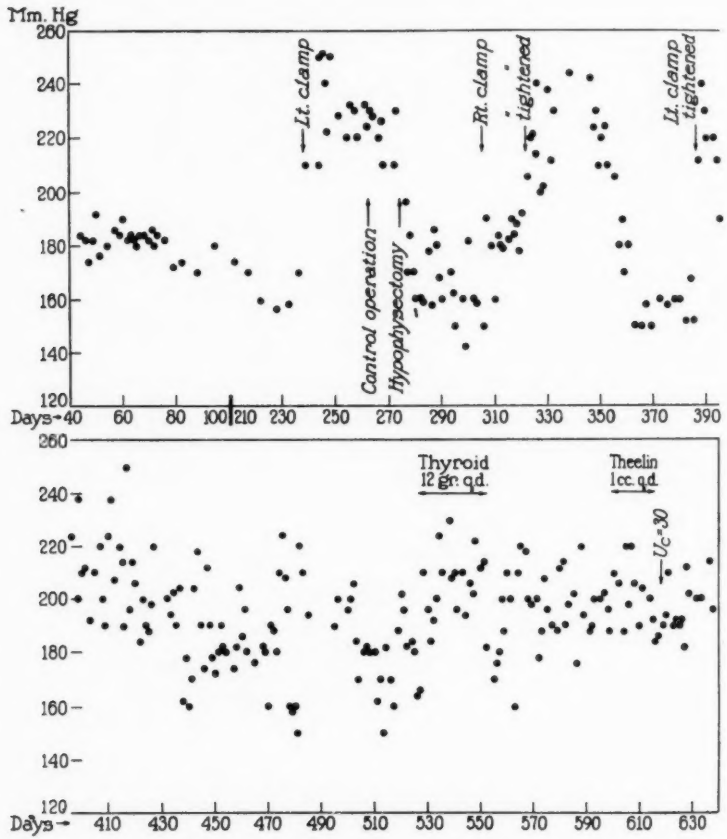

Fig. 3. Effect of control operation followed 12 days later by hypophysectomy on blood pressure of a dog with hypertension. Later both clamps were further tightened. The effect of thyroid feeding and theelin injection is also shown. The record covering a one hundred day period during which daily blood pressure measurements were made has been deleted from the chart of the preoperative control period.

and sometimes sustained rise in arterial pressure. These animals were clinically easily distinguishable from the first group. They were either lean or well-nourished, but not fat. They were active, alert, and in some cases excessively nervous. The blood pressure level was highly labile.

No change was observed in their coats. Polyuria was present less often. The basal metabolic rate of one of the dogs in this group was as high as +50 per cent of normal. However, due to the nervousness of the animal it is probable that the measurements were not made under basal conditions.

It should be noted that the general health of both of these groups of animals appeared to be excellent. Both male and female animals were in each group. The differences lay chiefly in their behavior.

Both groups of animals responded to large doses of thyroid substance by rise in arterial pressure. Thyroid was not administered until several months after hypophysectomy, so that time was allowed for the involutional changes in other organs to occur which result from removal of the pituitary gland. But the pressure never rose to the original high level even after feeding thyroid for 3 to 5 weeks. The dogs became very nervous and overactive but the rise was seldom greater than 80 mm. Hg. As a control, two normal dogs were given the same amount of thyroid as the hypophysectomized animals. The average blood pressure rise was not greater than 20 mm. in them. Theelin and antuitrin S produced no change in the average blood pressure level in either normal or hypophysectomized dogs.

Discussion. Hypophysectomy reduces the blood pressure of adult hypertensive dogs to levels which are either normal or somewhat above normal. The reduction usually occurs during the course of 20 days or less after operation. Trephining the skull and retraction of the brain, but without removal of the gland, does not lower the arterial pressure; consequently, the hypophysis appears to be responsible for the reduction of the blood pressure level. But if the pressure is lowered as a result of the hypophysectomy, it may be again elevated, if at times only temporarily, by tightening the clamps on the renal arteries or clamping the remaining renal artery if the hypertension has been produced by application of the clamp to only one artery.

We interpret these results in the following way. Hypophysectomy does not remove an organ which is secreting a pressor substance directly responsible for the hypertension following constriction of the renal artery. Were this so, it might be expected that the blood pressure would fall much more quickly than it does, and that the fall would be to normal, neither above nor below it. All of the animals should respond in essentially the same manner, and increase in the severity of the renal artery constriction after hypophysectomy should no longer produce a rise in blood pressure. Hypophysectomy performed a month or more before the renal arteries were constricted should prevent the hypertension ordinarily resulting from this procedure. The results were contrary to these expectations.

Removal of the hypophysis is well known to affect a number of organs

with which it is closely associated in normal metabolism. The chief among these are the thyroid gland, the adrenal glands, and the gonads. Atrophic changes with diminished function of these glands has been amply proven. Van Dyke (6) has recently reviewed this evidence. Administration of thyroid would thus be expected to aid in restoring the pressure to its elevated level. This was in fact observed, though the response was not usually great, even with doses of thyroid far greater than physiological. Among the remaining glands known to undergo involution after hypophysectomy, it would be anticipated that the adrenal gland would be most important in the genesis of hypertension because of the very low blood pressure which results from either disease or as a result of its extirpation. Indeed Goldblatt has very recently reported in a lecture (10) that bilateral adrenalectomy was followed by a sharp fall in blood pressure from the elevated to normal levels. One of us (I. H. P.) has confirmed this in 6 hypertensive animals.

It is, then, our opinion that most evidence favors the belief that hypophysectomy influences the arterial pressure level of hypertension in dogs indirectly through loss of its stimulating effects on other endocrine glands, chiefly the adrenal cortex and less importantly the thyroid. Whether other organs such as the liver are also affected is not known. It appears certain that the nervous system is not an essential link in the mechanism, for, as Freeman and Page (7) showed, hypertension persists after complete removal of the sympathetic nervous system, combined with denervation of the heart. Nor does section of the splanchnic nerves and removal of the lower thoracic ganglia affect the blood pressure (Goldblatt, Gross and Hanzal, 1). Furthermore, Glenn, Childs and Page (9) found that the blood pressure tends to return to elevated levels in hypertensive dogs even after complete destruction of the spinal cord below the fifth cervical segment.

Our experiments do not eliminate the possibility that injury to the diencephalic vegetative centers adjacent to the hypophysis may also have some influence on the response attributed to hypophysectomy. Indeed the fact that some of the dogs became excessively fat suggests that injury had occurred. One of us (I. H. P.) is investigating this problem in hypertensive cats.

We therefore conclude that hypophysectomy alters the reactivity of the vascular system by diminishing the activity of the adrenal cortex, thyroid and perhaps other organs in such a manner that it no longer responds as well to chemical stimuli resulting from constriction of the renal artery.

SUMMARY

1. Hypertension of the order of 240/160 mm. Hg was produced in dogs by constricting the renal arteries of dogs by means of Goldblatt's clamp.

It was maintained for several months. Hypophysectomy in these animals reduced the arterial pressure to levels slightly above normal (150/100 mm.) or below normal (90/40 mm.) within a period of 20 days. Hypophysectomy in normal dogs reduced the arterial pressure only slightly (from about 140/70 to 116/50 mm.).

2. Increasing the constriction of the renal arteries after hypophysectomy again produced a rise in blood pressure, but this tended to be less marked and transient, especially in the dogs which became sluggish and fat and exhibited reduced basal metabolism and often diabetes insipidus. The rise was better maintained in dogs which were thin and active and with normal or elevated basal metabolism.

3. After hypertension had been reduced by hypophysectomy, feeding thyroid (0.8 gram) raised the blood pressure moderately (190/120 mm. Hg), and injection of theelin (1 cc. daily) or antuitrin S (1 cc. daily) had no effect.

4. The effect of hypophysectomy on hypertensive dogs is believed to be an indirect one. It is postulated that the responsiveness of the blood vessels to chemical stimuli from the kidneys with constricted renal arteries is reduced. This may be due to lack of the secretions of the adrenal and thyroid glands. Deficiency of these secretions may in turn be due to withdrawal by hypophysectomy of the chemical stimuli normally afforded them by the hypophysis.

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CHANGES IN THE SUBMAXILLARY SECRETORY RESPONSE TO PILOCARPINE AFTER SECTION OF THE CHORDA TYMPANI

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It has long been an accepted fact that the submaxillary gland shows atrophic changes when its normal functional activity is abolished by section of the chorda tympani. According to Bradford (1888), the gland suffers a loss of one-third to one-half of its original weight within a few weeks after this operation, and from the reports of Vulpian (1878) and Langley (1885) it seems that the response of the gland to pilocarpine is also markedly reduced. These observations suggested to us that the rate at which atrophy occurs might be measured by studying the change in response to carefully regulated injections of pilocarpine given at appropriate intervals following denervation. To our surprise the expected decrease in response to pilocarpine failed to occur. On the contrary, the denervated gland secreted at a more rapid rate than the normal gland. Further investigation demonstrated that this curious change in the response of the submaxillary gland to pilocarpine after the severing of the parasympathetic fibers develops gradually and persists for months.

METHODS. Bilateral submaxillary fistulae were established in five dogs, following the technic already adequately described (Babkin, 1928). The animals were ready for experimentation three weeks postoperatively. No food was given for twenty-four hours before each test, but water was permitted *ad libitum*. Glass funnels were fixed to the skin around the fistulae with Mendeléeff's cement, and the saliva collected in 15 cc. graduated centrifuge tubes held in place by an elastic harness. The dogs were allowed to recline in a comfortable position on a laboratory table. No anesthetic was used and no restraining apparatus was necessary; the animals were undisturbed by the experimental procedures. Room temperature was adjusted to avoid panting. Salivary secretion was elicited by the intravenous injection of a 0.9 per cent sodium chloride solution containing 0.2 mgm. pilocarpine hydrochloride per cubic centimeter, the same strength of solution being used throughout. It was given into the short saphenous vein by a modified Colwell injection apparatus (Colwell, 1930). With this pump we were able to duplicate the pilocarpine

injections accurately in repeated tests on the same dog. A suitable rate of injection was determined for each animal and, once established, this was used in every test. The injection was continued for exactly twenty minutes in all experiments. The time at which the secretion started in each gland was noted and the amount of saliva in the collecting tubes was read every minute throughout the injection and in most experiments for ten minutes after the injection had been stopped. Except in the special instance noted below, an interval of at least one week was allowed to elapse between tests.

By use of the technic just described we first determined the response of the normal submaxillary glands to pilocarpine. The chorda tympani and chorda lingual nerves were then severed unilaterally in each animal (ether anesthesia) leaving the contralateral gland to serve as a further postoperative control. A section of the nerve approximately 1 cm. in length was removed at the point where the chorda tympani leaves the lingual to go to the submaxillary gland. The denervation was confirmed in each case by noting the absence of a reflex secretion from the operated side when lemon juice was placed on the animal's tongue. Soon after the operation and then periodically for several months, the secretory response of the denervated gland to the standard pilocarpine stimulus was observed and compared with the response of the contralateral normal gland.

EXPERIMENTS AND RESULTS. A. *Control experiments.* From the typical preoperative control experiment shown in figure 1A it may be seen that normally the response to pilocarpine is the same in the right and left submaxillary gland. Not only does the secretion appear at the same moment from both glands, but the total amounts collected during a thirty-minute interval are so nearly alike that the secretion curves are practically superimposable. Consequently, changes occurring in the response of one gland after its chorda tympani has been severed can be readily demonstrated by making a direct comparison with the simultaneous response obtained from the normally innervated gland.

B. *Experimental results after denervation.* The change which the cutting of the chorda tympani produces in the response to pilocarpine is well illustrated in figures 1C and 2B and table 1. It is evident from these tests, which were performed on the twenty-first day after the operation, that the denervated gland begins to secrete earlier and attains its maximal rate of secretion sooner than the normal gland. As a rule the denervated gland also secretes a larger volume of saliva than the normal (table 1). This change does not appear immediately after the chorda tympani has been cut. The results of tests made within four to six hours after the operation were essentially the same as those obtained before the nerve was severed. Even at twenty-two and forty-eight hours after the dener-

vation there was still no manifest difference in the secretion curves. But on the sixth day the denervated glands definitely began to secrete before the normal ones and also delivered more saliva. One of these experiments is shown in figure 1B. On the twenty-first day the difference in response

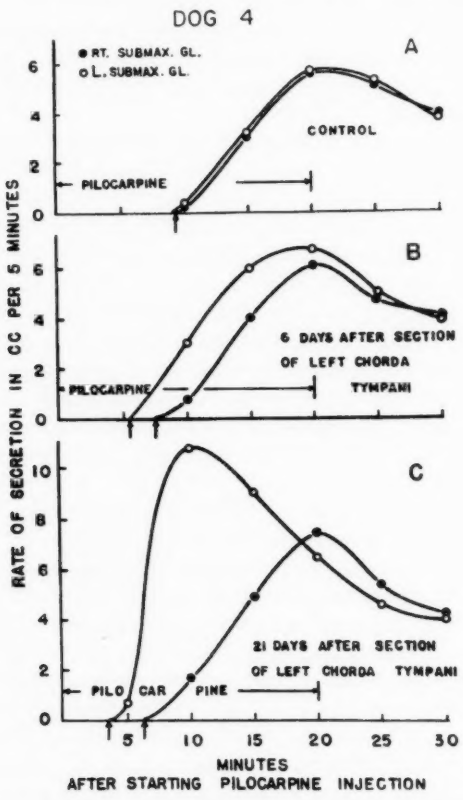

Fig. 1. Showing the simultaneous response of the right and left submaxillary gland of dog 4 to the continuous intravenous injection of a 2 mgm. per cent pilocarpine solution given at the rate of 37 cc. per hour for 20 minutes. A, control experiment. B and C, 6 and 21 days respectively after section of the chorda tympani.

was more pronounced, as may be seen by referring again to table 1 and figure 1C. The effect of the denervation on the increased response to pilocarpine is clearly a matter of gradual development, requiring two or three weeks for its full appearance.

After five and one-half to six months all five dogs were tested again.

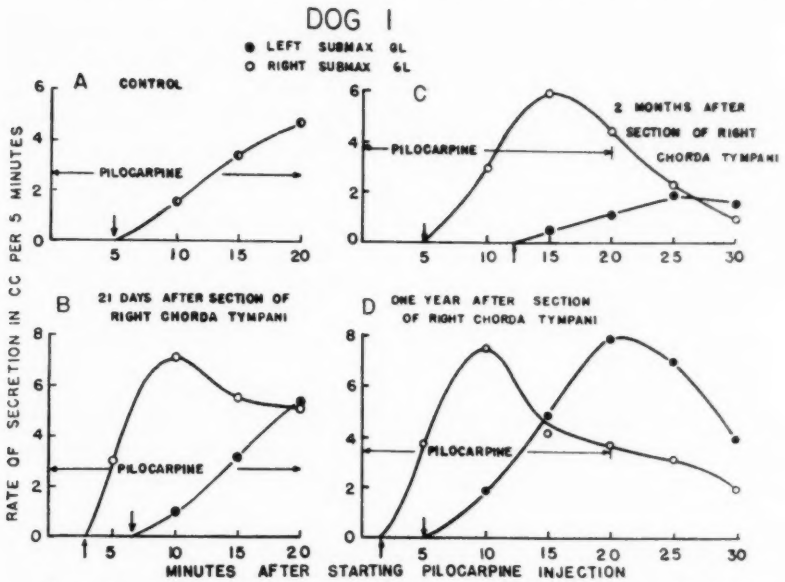
DOG 1
● LEFT SUBMAX GL
○ RIGHT SUBMAX GL
A CONTROL
PILOCARPINE
B 21 DAYS AFTER SECTION OF RIGHT CHORDA TYMPANI
PILOCARPINE
C 2 MONTHS AFTER SECTION OF RIGHT CHORDA TYMPANI
PILOCARPINE
D ONE YEAR AFTER SECTION OF RIGHT CHORDA TYMPANI
PILOCARPINE
RATE OF SECRETION IN CC PER 5 MINUTES
MINUTES AFTER STARTING PILOCARPINE INJECTION

Fig. 2. Showing the response of the submaxillary glands of dog 1 to the intravenous injection of a 2 mgm. per cent pilocarpine solution given at the rate of 37 cc. per hour for 20 minutes. A, control experiment. B, 21 days after the right chorda had been severed. D, same experiment one year after the tympani operation. C, result of the standard pilocarpine test following the injection of 1.25 grams of quinine hydrochloride (divided doses); two months after section of the right chorda tympani.

TABLE 1

Secretory response of the submaxillary glands to a 20-minute continuous intravenous injection of pilocarpine, 21 days after section of the chorda tympani on one side

DOG NUMBER	GLAND	TIME AT WHICH SECRETION APPEARS		SALIVA COLLECTED PER 5 MINUTES						TOTAL
				Minutes after starting injection						
				0–5	5–10	10–15	15–20	20–25	25–30	
		minutes	*seconds*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*
1	Left	6	30	0	0 9	3.1	5 4			9 4
	Right (chorda cut)	2	55	3 0	7 1	5 5	5 1			20 7
2	Left	8	45	0	0.4	1.4	3 6	3 5	2 5	11 3
	Right (chorda cut)	1	45	1 9	7.1	5 0	3 6	3 0	2 3	22 9
4	Left (chorda cut)	3	30	0.7	10 8	9.1	6 4	4 6	4 0	35 6
	Right	6	15	0	1.7	4.9	7 4	5 3	4 2	23 5
5	Left	5	10	0	1 9	4.7	5 6	4 0	1 8	18 0
	Right (chorda cut)	2	10	2 7	4 1	3 4	3 0	2 0	1 4	16 6

The difference in the secretion curves from the normal and denervated glands was still as striking as it was three weeks after the operation. Fig-

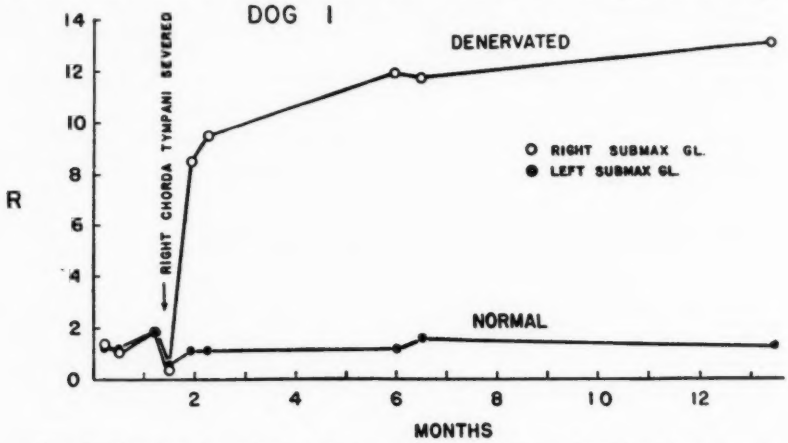

Fig. 3. Showing the values of R (see text and table 2) plotted against time in months before and after section of the right chorda tympani. R stands for the ratio of the amount of saliva secreted during the first 10 minutes after injection divided by the amount secreted during the second 10 minutes.

TABLE 2

Showing the change in R, $\frac{(\textit{Amount of saliva secreted in the first 10 minutes})}{(\textit{Amount of saliva secreted in the second 10 minutes})}$, *which results from section of the chorda tympani*

	DOG 1		DOG 2		DOG 3		DOG 4		DOG 5	
	Submaxillary gland									
	Left	Right	Left	Right	Left	Right	Right	Left	Left	Right
Preoperative controls	0.13	0.14	0.06	0.06	0.02	0.04	0.05	0.04	0.09	0.13
	0.12	0.10								
	0.20	0.20								
		Chorda tympani severed		Chorda tympani severed		Chorda tympani severed		Chorda tympani severed		Chorda tympani severed
4 hours postoperative			0.13	0.20	0.10	0.14	0.05	0.16	0.15	0.28
22 hours postoperative					0.17	0.21				
2 days postoperative	0.07	0.04								
6 days postoperative			0.06	0.18	0.09	0.25	0.08	0.22	0.15	0.54
2 weeks postoperative	0.11	0.85	0.08	1.04					0.18	1.45
3 weeks postoperative	0.11	0.96					0.14	0.74		
5½ months postoperative	0.10	1.20	0.14	1.16	0.18	1.49	0.10	1.10		
6 months postoperative	0.16	1.19							0.17	1.47
1 year postoperative	0.13	1.32								

ure 2D shows the result of a test on dog 1 after more than *one year.* Even at this time there appears to be no decrease in the response of the dener-

vated gland to the standard stimulus (compare with fig. 2B). It is interesting to recall the observations made by Guimarais (1932): that five months after the chorda tympani had been cut, the submaxillary gland could not be electrically excited but that it gave an abundant secretion in response to an injection of pilocarpine. He did not, however, compare the responses from the denervated and normal glands.

The change in the secretion curves brought about by severing the chorda tympani is particularly evident if one calculates the ratio of the amount of saliva delivered in the first ten minutes to that delivered in the second ten minutes after the beginning of injection. As the peak of the secretion curve from the operated gland occurs earlier, there is a striking increase in the value of this ratio, which we will call R. This is shown in figure 3 where the values of R for both the normal and operated gland of dog 1 are plotted against time in months. R increases rapidly within two or three weeks after the chorda tympani has been severed and is still rising one year after the operation. Examination of table 2 shows that similar charts could be constructed from the results obtained on the other four dogs studied.

Discussion. We have made some attempt to determine why section of the chorda tympani makes the submaxillary gland more sensitive to pilocarpine. We first considered whether or not it was due to an accumulation of preformed salivary substances (Anrep, 1920) in the inactive gland. This explanation was rejected on the basis of the following experiment: in four of the dogs in which the chorda tympani had been cut on one side two months previously, we tested, in the usual manner, the response to pilocarpine *immediately* after the glands had been activated by intravenous injections of acetylcholine (see p. 252, fig. 4); but under these conditions the secretion curves with pilocarpine were identical with those obtained from the unstimulated glands. If the peculiar secretory response of the denervated glands were the result of an abnormal accumulation of preformed salivary substances one would expect the acetylcholine to remove these substances and reduce the subsequent response to pilocarpine on the denervated side. These tests would perhaps have been more convincing had it been possible to activate only the denervated gland with acetylcholine. On the other hand, it should be remembered that a normal submaxillary gland may be repeatedly activated with a moderate stimulus without reducing the volume of saliva produced each time. Furthermore, in our experiments we could not see that the preliminary injection of acetylcholine had any effect on the subsequent response of the normal gland to the standard pilocarpine injection. That storage in the gland is of relatively little significance in these experiments is also indicated by the facts 1, that the volume of saliva produced is in every case far greater than the amount which could have

been stored in the gland, and 2, that the response to acetylcholine is greater on the normal than on the denervated side.

Because the secretory response to pilocarpine was greater and more prompt in the denervated gland, experiments were done to ascertain

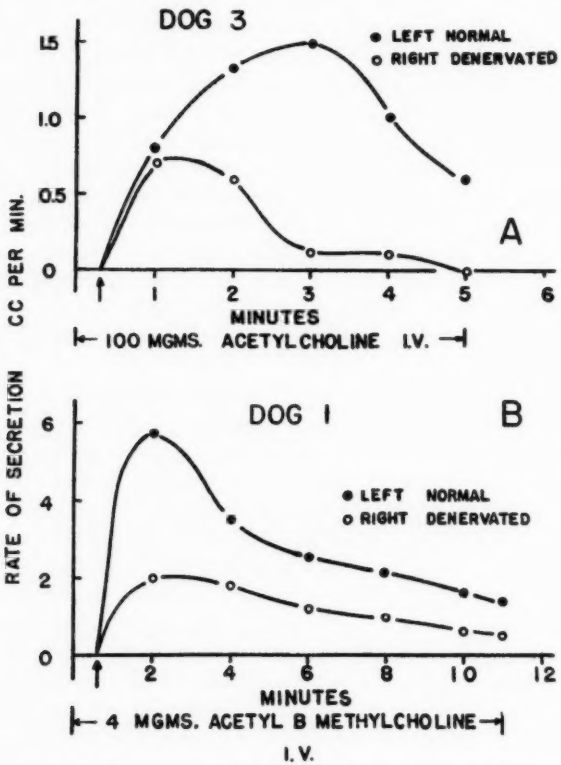

Fig. 4. A. Dog 3. Two months after section of the right chorda tympani. Showing that the denervated gland gives a smaller response to the intravenous injection of acetylcholine than does the normal, although secretion begins at the same time in both. B. Dog 1. Similar experiment with acetyl-β-methylcholine. Here again the denervated gland fails to secrete as much as the normal although the secretion begins at the same time in both (cf. figs. 1C and 2B).

whether this was possibly the result of vasodilatation following the cutting of the chorda tympani. Fröhlich and Loewi (1906) obtained results which they regarded as evidence for the presence of vasoconstrictor fibers in the chorda tympani, but more recent workers (Bayliss, 1908; Gesell, 1920; Dale, 1930) have disagreed with this conclusion. If there

are vasoconstrictor fibers in the chorda tympani which are responsible for limiting the response to pilocarpine on the normal as compared to the denervated side, then a maximal dilatation with amyl nitrite or nitroglycerine ought to make the response more nearly the same on both sides. To three dogs we gave amyl nitrite and to one nitroglycerine before and during the course of the usual pilocarpine injection. Blood pressure readings were not taken, but the heart rate was noted to be greatly increased, indicating that the drugs had been given in sufficient dose to lower the arterial blood pressure. We found no significant changes in the secretion curves except that both glands began to secrete more quickly. Changes in the vasomotor tone, therefore, appear to be of minor importance. Furthermore, if vasodilatation were concerned in producing the change in response to pilocarpine, the effect ought to occur immediately after denervation, whereas, as shown above, no measurable change appears for several days. Finally, if an increased blood flow were the determining factor, one would expect the denervated gland to be more sensitive to *any* parasympathetic drug, but this is apparently not the case (fig. 4A).

The gradual development of the change in response to pilocarpine after denervation suggested that possibly we were dealing with sensitization comparable with the increased sensitivity to adrenine evinced by smooth muscle after destruction of its sympathetic nerve supply (Meltzer and Auer, 1904; Meltzer, 1904; Elliott, 1905; Hampel, 1935). Hampel (loc. cit.), who followed closely the development of sensitization in the nictitating membrane of the cat, reported that during the first six to eight days after removal of the superior cervical ganglion, the response of the nictitating membrane to the same dose of adrenine increases rapidly. A more gradual increase takes place during the succeeding six to eight days, after which further change is slight. Superficially there is a similarity between these results and our observations on the submaxillary gland tested with pilocarpine. Pilocarpine is not, however, regarded as a true parasympathetic drug (Eddy, 1929; Baxter, 1932); and when the salivary flow in our dogs was tested with acetylcholine, the chemical mediator of parasympathetic action (Dale, 1929; Alles, 1934), there was no evidence of sensitization of the denervated gland. This was established in six experiments (two months after denervation of the gland), of which one is shown in figure 4A. In each case 100 mgm. of acetylcholine were injected intravenously over a period of four minutes. The secretion started at the same time from both glands and, contrary to the results with pilocarpine, the denervated gland always secreted less than the normal. The results of a test with acetyl-β-methylcholine (mecholyl) on dog 1, one year after section of the right chorda tympani, are shown in figure 4B. Four milligrams of this drug were given intravenously at a constant rate

over a period of eleven minutes. Here again the secretion appeared simultaneously from both glands and the denervated gland delivered less than half as much saliva as the normal. Since the increased response to pilocarpine after denervation could not be demonstrated with either acetylcholine or acetyl-β-methylcholine, we feel that it should not be looked upon as true sensitization of the gland.

Stavraky (1933) recently reported that in dogs under amytal anesthesia the response of the submaxillary gland to pilocarpine is increased after the parasympathetic nerve endings have been paralyzed by giving large intravenous doses of quinine, whereas under these conditions scarcely any secretion is obtained with either electrical stimulation of the chorda tympani or injections of acetylcholine. We were interested to know whether or not this increased response to pilocarpine was similar in character to the one obtained after degeneration of the preganglionic fibers in the chorda tympani. Three of the dogs were therefore given large doses of quinine hydrochloride (0.75–1.25 grams intravenously in 0.25 gram doses) during the hour preceding a standard test with pilocarpine. We were unable to confirm Stavraky's observations and found instead that the response to pilocarpine was depressed rather than increased; the effect was more pronounced in the normal than in the denervated gland (cf. figs. 2C and 2B). These results may not be surprising in view of the fact that quinine is regarded as a general protoplasmic poison (Cushny, 1928) and undoubtedly affects the gland cells as well as the nerve endings (Hunt, 1934). At any rate, the paralysis of the parasympathetic nerve endings with quinine did not abolish the characteristic difference between the responses of the normal and denervated glands to pilocarpine. This latter phenomenon, therefore, appears to have its origin in some change within the secretory cells which makes them more susceptible to the stimulating effect of pilocarpine.

It is apparent from figure 4A that our results with acetylcholine are not in accord with the general rule that "denervation increases the sensitiveness to stimulating agents" (Cannon and Rosenblueth, 1936). It should be noted, however, that in the experiments of Shen and Cannon (1936), which demonstrated sensitization of the denervated iris to acetylcholine, the peripheral neurones had been destroyed whereas in our experiment only the preganglionic fibers were cut. The difference between their results and ours might therefore be explained on the assumption that the peripheral neurones of the cranial autonomic become less sensitive to acetylcholine after denervation. But so far as we know this assumption is not supported by any evidence, and furthermore Cannon and Rosenblueth (1936) have shown that in the case of the peripheral neurones in the cervical sympathetic, denervation is followed by an increased sensitiveness to acetylcholine. At any rate, since our tests with

acetylcholine were carried out two months after denervation it is entirely possible that sensitization may have been present at an earlier period.

SUMMARY

In five dogs with double submaxillary fistulae the salivary response to pilocarpine was investigated before and after section of the chorda tympani. In each animal, only one submaxillary gland was deprived of its parasympathetic nerve supply, the other serving as a control.

The tests were carried out on the unanesthetized dog. A saline solution containing 2 mgm. per cent pilocarpine was injected intravenously at a constant rate (30–40 cc. per hour, depending upon body weight) for exactly twenty minutes. The rate of secretion was observed for thirty minutes.

In all control experiments the secretion curves from the right and left glands were identical (figs. 1A and 2A). Within a few days after its chorda tympani had been severed, the submaxillary gland responded more readily to pilocarpine; the secretion not only appeared more promptly but in greater amount than from the normal control gland (cf. figs. 1B and C). This effect appeared within six days (fig. 1B), developed fully in two to three weeks (fig. 1C and table 1) and was found undiminished six months or even one year after denervation (fig. 2D).

Evidence is presented which indicates that the increased response to pilocarpine of the submaxillary gland deprived of its parasympathetic nerve supply is not due either to a sudden release of preformed salivary substances or to a more abundant blood flow through the gland. Also it does not appear to be the result of sensitization of the parasympathetic nerve endings since the denervated gland responds less well to acetylcholine than does the normal (fig. 4A). The essential difference between the response of the denervated and normal submaxillary gland to pilocarpine is not obliterated when the parasympathetic nerve endings have been paralyzed with large intravenous injections of quinine hydrochloride (fig. 2C).

CONCLUSION

The secretory cells of the dog's submaxillary gland become more sensitive to stimulation with pilocarpine within a few days after the chorda tympani has been severed.

We wish to express our thanks to Dr. G. P. Heffner who assisted us in many of the early experiments.

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THE INFLUENCE OF THE FOREBRAIN ON AN AUTONOMIC REFLEX

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In a number of recent studies, concerned with the problem of the relationship between the forebrain and the autonomic nervous system, it has been shown that ablation of certain parts of the forebrain profoundly changes the degree of autonomic activity (cf. Bard, 1928) and that forebrain stimulation may excite or inhibit autonomic function (cf. Ranson, Kabat and Magoun, 1935; Hoff and Green, 1936). Little emphasis has been laid, however, on the quantitative estimation of the rôle of the forebrain in autonomic reflexes. The present investigation was therefore undertaken in an attempt to obtain more precise information by graphically recording certain autonomic reflex responses under varying conditions of stimulation and ablation of different forebrain areas.

The majority of indicators previously employed (e.g., the heart, blood pressure, bladder, intestines, etc.) either exhibit spontaneous activity, have a dual innervation, or are under the influence of several factors difficult to control simultaneously, and are therefore relatively unsuitable for the evaluation of central activity. The nictitating membrane (n.m.) of the cat has been shown to be almost uniquely free from such complications and was therefore chosen for most of the studies herein reported. Its responses to the stimulation of various afferent nerves have been described by Rosenblueth and Schwartz (1935) and Acheson, Rosenblueth and Partington (1936). Notable among the characteristics of its reflexes were the ease of their elicitation (single afferent volleys being often effective), their long after-discharge and the frequent appearance of positive rebounds. Slight modifications of these properties were easily detectable in the records and offered the possibility of a relatively detailed analysis.

METHODS. Cats were used throughout. In a few experiments they were decerebrated by the anemic method of Pollock and Davis (1930), but in the majority they were anesthetized with urethane (1.2 to 1.5 gm. per kgm. intravenously). Since the circulatory afferents in the vagi and carotid sinus nerves modify the reflex activity of the n.m. (Rosenblueth and Schwartz, *loc. cit.*), these nerves were routinely divided in the neck. In this procedure only occasional effort was made to spare the internal

carotid artery, as its presence or absence had no detectable influence on the results obtained. The adrenal glands were ligated in some of the experiments, but since they were found to play no significant rôle in the responses studied this step was omitted in most of the experiments involving long operative procedures on the central nervous system.

The sciatic nerves and occasionally the nerves from the liver were used as afferents for eliciting the n.m. reflexes. Shielded electrodes were applied to the cut central ends. As sources of current either a Harvard inductorium, a "multivibrator" or a set of condensers charged and discharged through a three-pole commutator were used.

The forebrain was approached through a trephine opening in the skull, made sufficiently wide with rongeurs to give easy access to the regions under investigation. The dura was opened and the cortex kept moist and warm by application of hot saline in the intervals between experimental procedures. The ablations were performed operatively with scalpel and spatula. For stimulation a pair of fine silver-silver chloride electrodes with an interpolar distance of approximately 1.5 mm. was employed. The current was supplied by a Harvard inductorium with 3 volts in the primary circuit.

The possibility that some of the effects recorded from stimulation of parts of the forebrain were due to spread of current was constantly borne in mind. Failure to get similar effects from structures within 2 or 3 mm. was ordinarily considered sufficient control. In doubtful cases the stimulated areas were functionally separated from their connections either with a sharp knife or by cocaine, without disturbing their position. Only if subsequent stimulation was without result was the original observation considered valid.

Isotonic contractions of the n.m. were recorded on a kymograph by means of a lever with a magnification of 1:10. Blood pressure was recorded from the femoral artery with a membrane manometer.

In six animals the neocortex was aseptically removed on one side and in five others on both sides in the manner described by Bard (1933). An interval sufficient for the degeneration of the cortico-fugal fibers was allowed in all cases before the terminal experiments. All these animals were in good condition and exhibited the behavior characteristic of such preparations previously described (cf. Bard and Rioch, 1937; Rioch and Brenner, to be published).

At the close of the experiments the brains were removed and fixed in formalin. The locations of lesions and electrodes were later ascertained by macroscopic section and inspection.

RESULTS. A. *Reflex responses of the n.m. in cats with intact central nervous system. Total extent of isotonic contraction.* As a first step in the further analysis of the normal n.m. reflexes a series of experiments was

carried out to determine the relationship between the recorded heights of the responses (R) and the intensity (I), or frequency (F) of the afferent

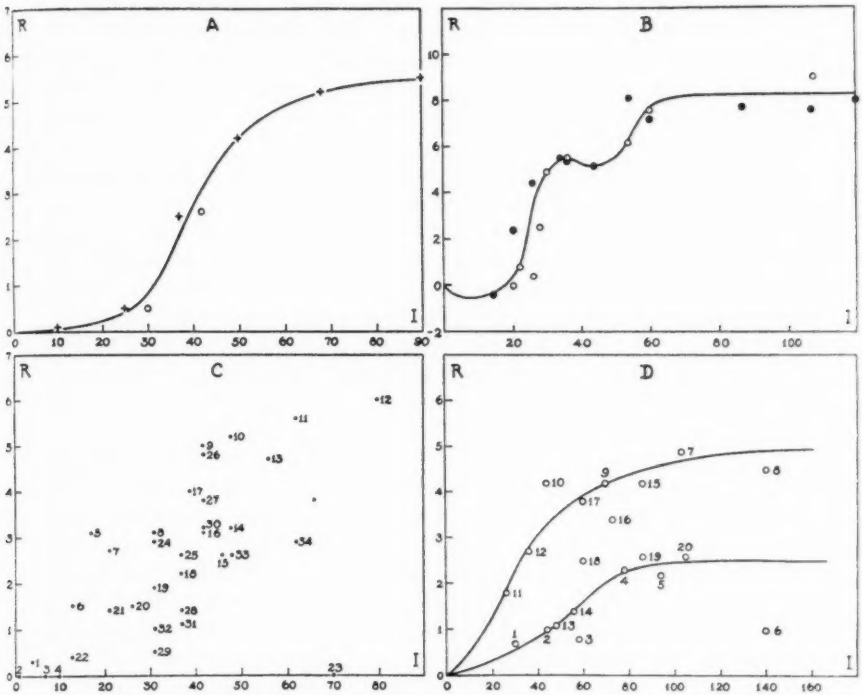

Fig. 1. Effects of varying the intensity of afferent stimulation on the reflex contractions of the nictitating membrane (n.m.) in various animals with intact central nervous systems. Adrenals ligated. In this and in the experiments illustrated by all succeeding figures, the animals were anesthetized with urethane and the vagi and carotid-sinus nerves have been interrupted. Ordinates: centimeters in the record. Abscissae: intensity in conventional units.

A. Simple curve of spatial summation. Liver nerves stimulated centrally. Frequency, 13 per second. Crosses: ascending; circles: descending.

B. Curve showing "break." Right sciatic stimulated centrally. Frequency, 2 per second. Circles: ascending; dots: descending.

C. Scattering of responses typical of many experiments (see text). Hamstring nerves stimulated centrally. Frequency, 25 per second. The numbers indicate the order of the observations.

D. Grouping of responses at two fairly definite levels. Liver nerves stimulated centrally. Frequency, 7.5 per second.

stimulation. The results may be interpreted as the effects of spatial and temporal summation respectively. The former will be given more detailed consideration here.

Consistent and reproducible relationships between I and R (F being kept constant) were obtained in occasional preparations. Such cases could be divided into two groups: 1, those in which plotting I (abscissae) against R (ordinates) resulted in smooth curves of an asymmetric S-shape (see fig. 1A); 2, those in which the curves thus obtained, although in general similar, showed one or more well-defined "breaks" (see fig. 1B). A further consideration of these two types of curves will be reserved for the discussion.

Most frequently, however, such experiments showed an extraordinary irregularity in the I to R relationship.

Although repeatable responses could usually be obtained if the frequency and intensity were held constantly at a rather high value, variations of either did not lead to consistent curves. The irregularities encountered seemed to be well outside the range of what might be considered "experimental error," sudden changes in the level of anesthesia, fatigue or the like. Figure 1C is illustrative of a not unusual instance. One experiment (fig. 1D), the clearest of fifteen, showed a grouping of responses at two rather definite levels and suggested that many of the variations encountered in other experiments might be attributable to the irregular participation of two or more separate "centers" in the reflexes studied. The numbers of the points in the figure refer to the sequence in which they were taken. It may be noted that the first five points fell fairly well on one smooth curve but the remaining points at distinctly different levels. By neglecting a few intermediate points, however, an entirely separate and higher curve might be drawn, representing the I to R relationship during this part of the experiment.

If temporal rather than spatial summation was tested similar results ensued, with the exception that when consistent F to R relationships were obtained the resulting curves were not S-shaped, but tended to fit tests for rectangular hyperbolas. Most frequently, however, random scattering was encountered similar to that found in the experiments on spatial summation.

In many of the experiments in this series, but particularly in those in which no consistent I to R or F to R relationships were found, the response of the n.m. to the first stimulus applied to the afferent nerve was proportionately much greater than its responses to the succeeding stimuli at intervals of three to ten minutes. When the preparation was allowed to remain quiescent for thirty to forty-five minutes or longer, the large response could be elicited once again. In these primary responses the greater central activity was indicated by increased duration of after-discharge as well as by the greater total height of the response.

After-discharge. The duration and degree of activity and mode of cessation of after-discharge showed as marked irregularities as did the total

heights of the responses. In general, after-discharge was greater both after the larger responses and also, irrespective of the height of the responses, after prolonged stimulation. The variability, however, was too marked to allow a quantitative formulation on the basis of known factors. The after-discharges of long duration tended to disappear in an irregular manner, while the degradation of shorter after-discharges followed smooth curves. Striking instances of long, irregular after-discharge might suddenly appear in the midst of a series of quite conventional responses evoked by repetition of a given stimulus. This occurred most frequently with stimuli of relatively high intensity and frequency in preparations not too deeply anesthetized. Sometimes there was concomitant increase in activity of the skeletal muscle reflexes, but that these phenomena were not necessarily related was shown by experiments such as that shown in figure 3 in which no reactions of the skeletal muscles could be seen. Certain features in the observations illustrated here were of particular significance. The n.m., after a small, preliminary relaxation at the end of stimulation, remained constantly contracted for three minutes, and then suddenly relaxed to the original resting level in a manner similar to the usual termination of a short after-discharge. The next succeeding response (fig. 3B) showed a greatly reduced after-discharge, and the total height was not only less, but consisted largely of excitatory rebound, the initial response being relatively insignificant.

Excitatory rebound. Variations in the extent of excitatory rebound were encountered in different animals and at different times in the same animal. For convenience two general types of rebound have been defined on the basis of the proportions of the initial and rebound components, respectively, in the total height of the response. In type 1 have been included those cases in which the initial response was small and came to a constant level during the first few seconds of stimulation, followed by a rapid rebound which was frequently several times larger than the initial response (fig. 4A). In type 2 the initial response continued slowly to a higher level and the rebound on cessation of stimulation was proportionately less, though similar in rate and form to that in type 1 (fig. 6A). Type 1 rebounds were more frequent with moderately deep anesthesia and high intensity of stimulation (secondary coil of Harvard inductorium at 4 to 6 cm.).

Inequality of responses on the two sides. In 16 preparations in which simultaneous records were taken from both nictitating membranes, 4 gave equal responses on the two sides, 6 showed preponderance of the response from the left and 6 from the right n.m. The inequality was unaffected by changing the side of afferent stimulation (cf. Rosenblueth and Schwartz, 1935), or by altering the position of the animal with respect to the table or to gravity. That this phenomenon was due to central activity and

not to differences in mechanical advantage of the recording apparatus or to peripheral nerve injury was indicated by the following observations. The effect was not constant and a marked difference in laterality for the first few responses in an experiment occasionally gave way to approximate bilateral equality. In several cases the inequality was much less marked on central (cortical or sub-cortical) stimulation than in response to sciatic stimulation. Finally, and possibly most significantly, there were some preparations in which, although the total heights of the responses of the two membranes were approximately equal, the after-discharge was prolonged on one side and short on the other.

B. *Electrical stimulation of the brain.* It was evident from the results recorded above that the neural mechanism responsible for the sciatic-n.m. reflexes in urethanized cats was complex and possibly involved several different regions in the brain. In order simply to determine the localization of such possible "centers" the forebrain was explored by means of stimulating electrodes, with the results described below. It should be noted, however, that the elicitation of responses by electrical stimulation of a given area is not proof of the participation of that area in reflex activity. Such proof can only be obtained by methods of ablation (see Bard and Brooks, 1932).

Effects of electrical stimulation of the cerebral cortex on nictitating membrane reflexes. In animals lightly anesthetized stimulation of moderate intensity (coil distance 8 to 9 cm.) applied in either one of two rather well-defined areas evoked small contractions of the n.m. One of these areas comprised the middle thirds of the lateral and the superior ectosylvian gyri, the other the depths of the cruciate sulcus and both its margins (see fig. 2). Deeper anesthesia usually abolished these responses, but if a background of reflex activity was produced by sciatic stimulation marked increases in contraction were always easily elicited by excitation of the designated cortical areas, greater effects being usual from the regions neighboring the cruciate sulci. That this constituted a true "facilitation" is illustrated in figure 5, which shows the results of combining a sciatic with a cortical stimulation, both of them in themselves subliminal. In general, small responses to afferent stimulation were relatively more facilitated by cortical excitation than were large ones. Sometimes (see fig. 4B) cortical stimulation interposed in the course of a response produced by continuous tetanization of the sciatic brought about a sudden increase in contraction comparable to the rebound to be expected on cessation of sciatic stimulation (fig. 4A). After-discharge of cortical effects so produced frequently lasted until cessation of reflex stimulation, i.e., the reflex contraction tended to stay at the facilitated level (figs. 4B, 7B). In other instances, especially on stimulation of the rostral part of anterior sigmoid gyrus, after-discharge did not appear or was masked by inhibition (fig. 7C).

Marked inhibition, uncomplicated by a preceding facilitation (figs. 7A, 9), was routinely obtained from the extreme frontal areas of the cortex, including the ventral half of the gyrus proreus, the cortex surrounding the presylvian sulcus and the gyrus orbitalis (fig. 2).

Although such inhibition could occasionally be demonstrated on the background of "tonic activity," it was usually necessary to increase this activity by stimulating an afferent nerve before good inhibitory effects could be produced. Reflex after-discharge, however, frequently supplied a background adequate for the demonstration of clear-cut inhibitory effects. In these cases the cessation of cortical stimulation was followed by a return of the contraction only to the level which would have been reached by the continuously declining after-discharge (fig. 10), and not to the original level as when the sciatic was stimulated persistently (fig. 9). After-discharge of inhibition was much shorter than excitatory after-

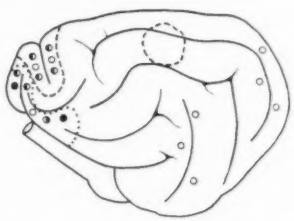

Fig. 2. Regions of the cortex, stimulation of which yielded changes in the reflex contraction of the nictitating membrane or in the blood pressure. Areas outlined in broken lines facilitated the response of the n.m.; that outlined in dots yielded inhibition. Circles denote areas affecting blood pressure. Fully-black indicates facilitation; right-half black, facilitation followed by inhibition; left-half black, inhibition followed by facilitation; blank circles, pure inhibition.

discharge. This was also demonstrated by experiments in which stimulation was applied to an inhibitory cortical area alone and immediately followed by an excitatory afferent stimulus. The contraction of the ipsilateral n.m. was slightly reduced by the preceding inhibitory stimulus but practically no effect was seen on the contralateral side. If the same stimuli were given coincidentally (figs. 11A and B), all reflex effects were blocked until after the cessation of stimulation, when the longer after-discharge of the reflex became immediately apparent as an excitatory rebound. If the inhibitory stimulus was interposed during the course of a reflex contraction, it was less effective than if it was started coincidentally, i.e., it prevented the appearance of contraction more effectively than it abolished an existing contraction. Also noteworthy is the fact that such a procedure did not affect the total height of the response; immediately after the cessation of cortical stimulation the build-up of excitation resumed at a rate greater than before inhibition was interposed, and the contraction reached

the same height it would have attained without interference. The records shown in figures 10 and 11 were from a chronically hemidecorticate cat. Similar, but less striking, results were usual in normal cats.

When contraction of the nictitating membrane was evoked by bipolar stimulation of the lateral and posterior hypothalamic areas, instead of reflexly from the sciatic, inhibitory phenomena similar to those described above were obtained from cortical stimulation.

Laterality. The effects, whether excitatory or inhibitory, were ordinarily bilaterally equal, but in some experiments differences between the effects on the two membranes were large enough to be significant. An appraisal of cortical heterolaterality was, of course, complicated by the fact that the responses of the two n.ms. to afferent stimulation were frequently unequal (cf. p. 261) and, in fact, the differences in the effect of cortical stimulation found in six of eleven instances were probably to be explained on this basis. In the remaining five, clear evidence of a preponderating influence of the contralateral inhibitory cortex was found. Facilitation was more marked ipsilaterally in four, and was equal on the two sides in the fifth. On the other hand, in the three hemidecorticate animals tested (one having had the right and two the left cortex ablated 3 months previously) some inhibitory effects were more marked on the ipsilateral side. Facilitation showed no differences between the two sides.

Effects not cortico-cortical. In several experiments an attempt was made to determine whether the results obtained could be attributed to a cortico-cortical effect. The results were entirely negative; surgical separation of the inhibitory from the excitatory area did not significantly alter the function of either.

Stimulation of sub-cortical areas. Macroscopic examination of the formalin-fixed brains of the animals surviving unilateral and bilateral removal of the neocortex showed that the ablations were roughly similar in the hemispheres involved. The neo-cortex in most cases was entirely removed, but in some a small fragment of the gyrus splenius posterior or of the gyrus orbitalis remained. The olfactory brain was intact with the exception of occasional slight lesions of the lateral margins of the olfactory stalk, of the pyriform lobe and of the hippocampus. In all cases the septum was considerably shrunken. In most of the preparations the lateral margin of the putamen and in some the rostro-lateral margin of the head of the caudate nucleus were involved in the lesion. Invariably, however, the head of the caudate was smaller than normal and the thalamus, though never operatively injured, was small and shrunken. The remaining portions of the lateral and third ventricles together with the foramen of Monroe were widely dilated, more markedly so in the bilateral than in the unilateral ablations.

On dissecting the head at the beginning of the terminal experiments, a

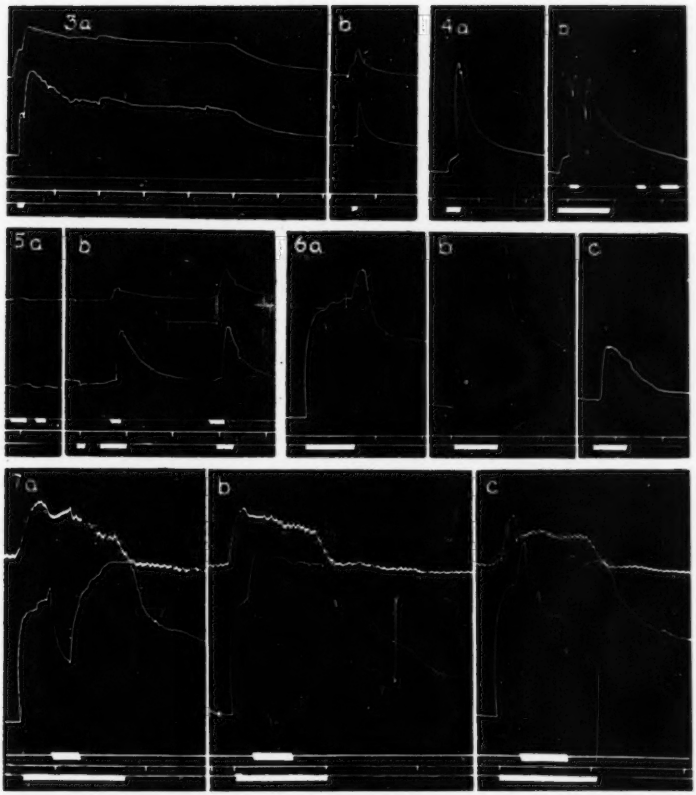

Fig. 3. Successive responses of both nictitating membranes to the same stimulus. Upper record, left n.m.; lower record, right. Middle signal, 30-second intervals.

A. At lower signal right sciatic stimulated centrally; coil distance, 8 cm.

B. Same 3 minutes later (see text).

Fig. 4. Comparison of excitatory rebound at end of stimulation with facilitation produced by cortical stimulation. Skull open.

A. At lower signal, right sciatic stimulated centrally; coil distance, 8 cm.

B. Same, but anterior sigmoid gyrus stimulated at upper signal. Note ineffectiveness of cortical stimulation except during sciatic excitation.

Fig. 5. Effect of combining an afferent stimulus with a cortical stimulus, neither of which gives significant effects alone. Upper record, left n.m.; lower, right. Upper signal, stimulation of left anterior sigmoid gyrus; coil distance, 10 cm. Middle signal, 30-second intervals.

Fig. 6. Effect of sections at different levels in the cerebrum on the reflex response of the n.m. Skull open. At signal, right sciatic stimulated centrally. Coil distance, 6 cm. Time, 30-second intervals.

A. Central nervous system intact.

B. After removal of all cortex anterior to and including the motor areas.

C. After intercollicular section.

Fig. 7. Effects of cortical stimulation on the reflex contraction of the nictitating membrane and on blood pressure. Upper record, blood pressure; lower record, right n.m. Lower signal, right sciatic stimulated centrally; upper signal, stimulation of cortex.

A. Pure inhibition of n.m. Slight inhibition of b.p. Margins of gyrus proreus stimulated.

B. Pure facilitation of n.m. No effect on b.p. Anterior margin of cruciate sulcus stimulated.

C. Mixed facilitation and inhibition of n.m. Pure inhibition of b.p. Stimulation of cortex midway between A and B.

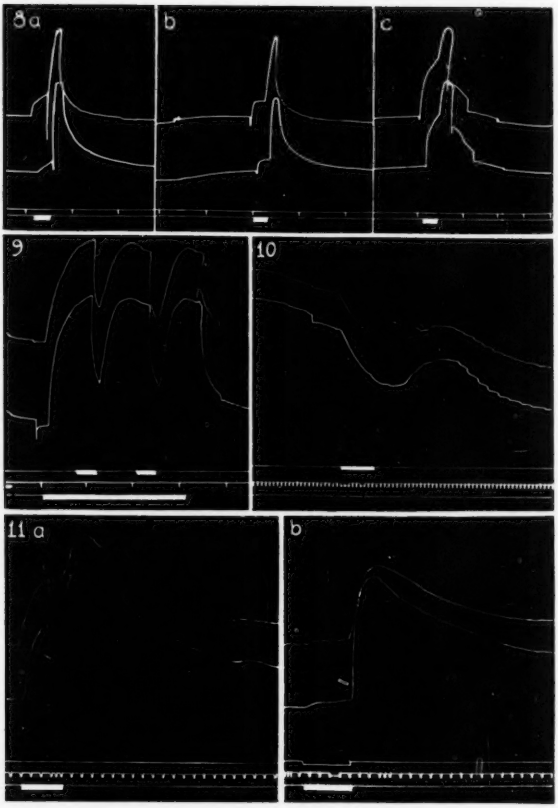

Fig. 8. Type 1 rebound reduced to type 2 by cortical ablation (see p. 268). Skull open. At signal right sciatic stimulated centrally; coil distance, 6 cm. Time signal, 30-second intervals.

A. Central nervous system intact.

B. After removal of cortex anterior to left cruciate sulcus.

C. After removal of same area on right side.

Fig. 9. Example of equal inhibitory effects from right or left cortex on both nictitating membranes. Upper record, left n.m.; lower record, right. Lower signal, interrupted tetani applied to right sciatic; coil distance, 7 cm. First upper signal, margins of right presylvian sulcus stimulated; coil distance, 8 cm. Second upper signal, same left.

Fig. 10. Inhibition of after-discharge. Right cortex removed 3 months previously. Upper record, left n.m.; lower record, right. Ninety seconds before beginning of record, right sciatic stimulated for one minute. At signal, margins of remaining gyrus proreus stimulated; coil distance, 7 cm. Middle signal, 1-second intervals.

Fig. 11. Complete inhibition of sciatic nictitating membrane reflex during simultaneous stimulation of inhibitory cortex, with rebound after cessation of both peripheral and central excitation. Right cortex removed 3 months previously. Upper record, left n.m.; lower record, right. Upper signal, cortical stimulation; coil distance, 8 cm. Lower signal, right sciatic stimulation; coil distance, 10 cm. Middle signal, 2-second intervals.

dense layer of scar tissue was found under the temporal muscle. This was adherent to a greatly thickened dura which was in turn adherent to the cut surfaces of the brain. The uninjured ventricular surfaces (e.g., head of caudate, third ventricle, fornix, fimbria and hippocampus) were free and allowed a relatively exact application of the stimulating electrodes.

Very strong contraction of the nictitating membrane was evoked from the posterior half of the hypothalamus, from the lateral hypothalamic area, and from the zona incerta. Good facilitation of the sciatic-nictitating membrane reflex was obtained in addition by stimulation of the rostral part of the periventricular system, the anterior hypothalamus, the caudal half of the septum, the tuberculum olfactorium and the pyriform lobe. Occasionally a small contraction of the n.m. was elicited from all these areas except the last without the facilitating sciatic stimulation. In only one instance in four preparations with both n.ms. recording was there a difference in the effects on the two sides. In this case stimulation of the tuberculum olfactorium resulted in a larger contraction of the ipsilateral membrane.

In contrast with the strong excitatory effects noted above, inhibitory effects were weak and were elicited from only three regions. In five preparations, including three in which no trace of the gyrus orbitalis remained, slight inhibition of the sciatic-n.m. reflex was repeatedly obtained by stimulation in the region of the rostral pole of the amygdala. Similar results were obtained in four preparations from the rostral, basal part of the septum, but the extent of the region responsible was not sharply defined and varied considerably. Finally, in one cat definite, but weak, inhibition resulted from stimulation of the rostral wall of the third ventricle, though good facilitation was evoked from points 2 to 3 mm. caudad.

Further evidence for the presence of weak inhibitory mechanisms was furnished by the rare occurrence of rebounds. These were always excitatory and were noted on a few occasions at the cessation of stimulation of the basal, caudal portion of the septum and, once, of the lateral margin of the zona incerta.

Occasional small responses, excitatory and inhibitory, were obtained from scattered areas, including the head of the caudate, the fornix, the hippocampus, the dorsal surface of the thalamus, etc. These were not reproducible in the same or in succeeding preparations, though carefully investigated. No adequate explanation was found, but the weight of evidence favored the view that the areas mentioned were not involved in the n.m. reflexes in these preparations.

C. *Effects of lesions in the c.n.s. Cortical lesions.* After ablation of various amounts of cortex, which always included all cortical tissue anterior to the ansate sulcus, temporal and spatial summation (cf. p. 258) regularly followed smooth and consistent curves in contrast to the rarity

of their appearance in cats with intact c.n.s. This was not due to a mere depression of all responses by the operative procedure, because the maximal height of response was not reduced. Further assurance that transitory effects were not involved was gained from an experiment on an animal which had had the cortex removed 5 months previously.

Certain differences in the form of individual responses were also found in cats with cortical damage. Particularly noticeable changes were produced in the excitatory rebound. Thus, although type 1 rebounds (cf. p. 261) were frequently seen in normal animals, they never appeared in cats with acute injury to extensive parts of the cortex, nor were they found in the five chronically decorticate preparations. In one of these a rebound intermediate between the very marked type 1 and the small type 2 was seen. At autopsy a remnant of the gyrus orbitalis was found on both sides. Even the slight depression of the cortex incident to opening the skull without opening the dura seemed in some cases, at least, to prevent the appearance of type 1 rebounds. In three animals rebounds of this sort were obtained after the dura was opened, and in these ablation of only the cortex anterior to both cruciate sulci was followed by increased initial response to stimulation with proportionately less rebound, type 2 (fig. 8). Finally, all cortical tissue posterior to the motor area was removed aseptically in two stages. Two weeks after the last operation, placing and hopping reactions were normal on one side and classed as fair on the other, thus showing that the remaining cortex was functional (Bard, 1933). This preparation gave the type 1 rebound typical of normal animals. Further evidence that the frontal poles were very important for the production of the excitatory rebounds was contained in the following experiments. Type 2 rebounds were encountered in 12 animals, many of which had had large areas of cortex posterior to the cruciate sulcus removed; removal of the remnants was followed by complete disappearance of the rebound in five cases (fig. 6B) and definite reduction in five more. Only two cases exhibited no change. The disappearance of the rebound was in all cases due to an increase in the height of initial response during stimulation; the total height of the response (i.e., initial, plus rebound) remained the same or increased. A few cases in which the total height was depressed were not considered in the series.

The evidence that areas of the cortex anterior to the cruciate sulcus tend to decrease the initial response of the n.m. to afferent stimulation and to allow the appearance of positive rebounds may therefore be summarized as follows. Marked type 1 rebounds were always reduced by ablating only this region. Type 2 rebounds and type 1 rebounds in one chronic preparation were not significantly affected by lesions involving all neocortex except that rostral to the ansate sulcus but were reduced or abolished in ten out of twelve instances by ablating these anterior re-

gions. Furthermore, the appearance of excitatory rebound may be regarded as evidence for the presence of inhibition during stimulation. As shown above, cortical inhibition of the n.m. was only found in the most rostral parts of the cortex, areas contained in the tissue, removal of which reduced the inhibitory effects of afferent stimulation and *pari passu* the relative extent of excitatory rebound.

Other changes were less frequently observed after removal of the areas anterior to the cruciate sulci. One which occurred often enough to be of significance was an increase in the basal level of contraction immediately after ablation of the inhibitory cortical areas.

Long irregular after-discharges of the type described on p. 260 were not seen in the chronic decorticate preparations nor in any of the animals with acute injury to the frontal cortex. Such after-discharge was, however, repeatedly recorded in an animal which had had all cortex posterior to the motor area removed 2 weeks previous to the experiment.

Lower sections. Ablation of the olfactory brain and striatum, in addition to the neo-cortex, in acute experiments on one of the decorticate preparations and on several normal animals resulted in no further modification of the sciatic-n.m. reflex. Electrolytic lesions which destroyed large parts of the parolfactory nuclei, tuberculum olfactorium and pre-optic area were likewise without noticeable effect in most instances.

In all animals with bilateral lesions involving the anterior hypothalamus, however, as well as in a few of the preparations described in the preceding paragraph (possibly with trauma of the hypothalamus not anatomically demonstrable) the rebound disappeared completely. The initial responses in these cases either rose moderately rapidly to the original total height or were of the decerebrate type (see below). It should be noted here that the difficulties encountered in these experiments were increased by the fact that in cats under urethrane anesthesia respiratory failure frequently followed premammillary transection of the brain stem and almost invariably followed postmammillary (Sherrington) decerebration. The results reported here and in the following paragraph were obtained on animals in which spontaneous respiration continued, or in which the transection was performed under ether with subsequent immobilization with curare and artificial respiration. Similar responses of the n.m. were obtained by both methods.

Transection of the brain stem at or below the level of the mammillary bodies produced an abrupt change in the responses. The rise was more gradual, the total height was greatly decreased regardless of the duration of stimulation, all rebound disappeared and there was little or no after-discharge (fig. 6C). In these preparations sciatic stimulation in the form of a series of short tetani resulted in a contraction of the n.m. similar to that evoked by a single long tetanus, whereas, in all the preparations

described above, in which inhibition could be otherwise demonstrated, a properly timed, series of tetani resulted in a moderately slow, smooth contraction of the n.m. to a total height greater than that elicited by an uninterrupted tetanus.

Sections below the posterior margin of the pons made either by surgical section or ligation of the basilar and carotid arteries (Pollock and Davis, 1930) completely abolished the sciatic-n.m. reflex in urethanized animals. A few experiments on chronic spinal animals were performed, but the results did not justify conclusions as to whether the n.m. has spinal "centers" similar to those so clearly demonstrated by Brooks (1933) for other autonomic reflexes.

D. *Blood-pressure responses.* Since the sympathetic division of the nervous system has been shown to respond more or less as a unit in certain emotional states, it is of interest to ascertain whether the same was true under different experimental conditions (cf. Cannon, 1936). For this purpose the b.p. was recorded synchronously with the n.m. responses in several normal cats and in three of those with bilateral removal of the neo-cortex.

Peripheral afferent stimulation. The reflex responses of the n.m. to peripheral afferent stimulation contrasted sharply with those of the b.p. The latter typically consisted of a rapid smooth rise, followed, on cessation of stimulation, by a moderately strong *inhibitory* rebound, sometimes a second, slight rise, and finally return to the original level. Opposite effects on the n.m. and b.p. were also readily obtained by varying the intensity and frequency of stimulation (Rosenblueth and Schwartz, *loc. cit.*).

Cortical stimulation. Electrical stimulation of certain areas of the cerebral cortex, both with and without a background of reflex excitation, elicited rises or falls in the b.p. The cortical areas involved and the type of response have been illustrated in figure 2. In certain cases opposite effects on the n.m. and b.p. were evoked from the region of the presylvian fissure and the orbital gyrus. The presence of rebound phenomena in the b.p. response from these areas as well as from the anterior sigmoid gyrus indicated the presence of both excitatory and inhibitory mechanisms. These results, considering the differences in the experimental conditions, were in good agreement with those reported by Hoff and Green (1936). The chief discrepancies were the elicitation of inhibition from the occipital area and the failure to do so from the lateral gyrus in the present investigation.

Subcortical stimulation. Although in most instances of subcortical stimulation the responses of the n.m. and b.p. were in the same direction, that is, contraction and relaxation of the former accompanied by rise and fall respectively of the latter, it was rare that the effects on the two systems were quantitatively equal. In some cases one system did not respond at

all, while the other showed pronounced excitation or inhibition. Contractions of the n.m. were well maintained during stimulation, but relaxation poorly, whereas fall of b.p. was better maintained than rise. On several occasions positive rebounds of the n.m. were accompanied by no or even negative rebounds of the b.p. and vice versa. The most striking discrepancies were obtained in three cases when the electrodes were applied to a region bordering one or other of the chief inhibitory areas. As noted above for the n.m., the b.p. was most consistently and strongly inhibited from the rostral-basal part of the septum and from the region of the rostral pole of the amygdala. In one cat from a point just lateral to the former, and in two cats from points just caudal to the latter, contraction of the n.m. and fall in b.p. were evoked as initial responses.

Discussion. The results reported in section A, p. 258, point definitely to the conclusion that the output of the "centers" involved in at least some of the reflex activity of the autonomic nervous system does not bear any constantly discernible relationship to the frequency or intensity of the afferent stimulus.

Exceptional experiments on normal animals in which consistent relationships between stimulus and response occurred, when considered together with the regular finding of such relationships in decorticate animals suggest that one center at least behaves in a fairly simple and predictable way. It is probable that its output bears a roughly lineal relationship to the frequency of afferent impulses. That the F-R curves are hyperbolic can be explained by the relationship existing between efferent frequency and the height of effector response (cf. Rosenblueth, 1934, for heart rate). Strict lineal comparison of the hyperbolas obtained by afferent and efferent stimulation was not possible owing to the frequent existence of a threshold frequency of afferent stimulation which, though small, prevented the curves from passing through the origin. The explanation of this threshold must await further work, but it is very probably related to the existence of inhibitory fibers in the available afferent nerves.

The S-shape of the curves found for spatial summation is familiar in other similar systems and was almost certainly a reflection of the random distribution of thresholds in the fibers under the stimulating electrodes. The break in the curves sometimes encountered at moderately high intensities suggests the existence of a relatively small group of inhibitory fibers with a narrow distribution of thresholds. Such an interpretation is consistent with the fact that relatively high intensities of tetanic stimulation most frequently produce small initial responses with large excitatory rebounds.

The wide scatter of responses so frequently encountered in other experiments could not, however, be completely due to a mixture of afferents since the threshold changes necessary on such a basis would have been too

great and too sudden. A much more likely explanation is found in the postulation of abrupt changes in the "set" of the "center." That these changes might be due to the activity of other "higher centers" is the conclusion drawn from the evidence presented above, namely, that measures taken to depress such "higher centers" (principally the cortex in these experiments) simplified the reflex responses, and made possible the obtaining of smoother, more consistent curves for temporal and spatial summation.

Apparently the "secondary centers" did not influence the lower centers in a graded manner, but entered in a roughly all-or-none fashion. Experiments in which responses group about two definite levels, such as that illustrated in figure 1D, certainly suggested that such may have been the case. Once a center was excited to the point of participation it tended to remain within reach of the afferent, sometimes for considerable periods, as witness points 7, 8, 9, 10, 11, 12 in figure 1D, and then dropped out for some as yet unknown reason. Under appropriate conditions it may be possible actually to record the immediate effects of such a dropping out of continuously sustained activity on the part of a relatively large group of neurones. That interpretation would seem to apply to the phenomenon reproduced in figure 3, in which the after-discharge continued almost unchanged for 3 minutes and then suddenly disappeared. After-discharges of this sort were relatively common in cats with intact c.n.s., though never consistent in any one preparation. They have not been seen in either acute or chronic decorticate cats, and suggest a speculative correlation with the well-known observation that the rage responses of decorticate cats end shortly after the cessation of noxious stimulation.

Further evidence for the complex nature of the central mediation of these reflexes was found in considering the variations of the excitatory rebounds encountered. The generally accepted explanation of such rebounds is that afferent stimulation involves both inhibitory and excitatory fibers, but that the inhibition produced disappears rapidly and allows the more persistent central excitatory state to become apparent. Most of the evidence here presented is consistent with such a view. Particularly relevant were the experiments in which rebounds were produced by stimulating an afferent which gave a predominantly excitatory response while simultaneously stimulating an inhibitory cortical area (fig. 11). Cessation of the two stimuli was followed by a clear-cut positive rebound similar to those obtained on other occasions solely by stimulation of an afferent which contained a large inhibitory component. Significant also were the other experiments on central inhibition which demonstrated its relatively short after-discharge (figs. 10 and 11). A rather nice balance between inhibition and excitation seemed to exist in those animals in which sciatic stimulation led to a small initial response with a large positive rebound, for a slight addition to the excitatory component brought about by

cortical stimulation, as in figure 4B, enabled the responses to break away from the inhibition. It was not surprising, therefore, that very slight changes in the inhibitory component such as the minor trauma incident to opening the skull over the inhibitory cortical areas, were sufficient to increase the initial height of the responses and *pari passu* decrease the rebound.

The evidence presented from the ablation experiments (section C) allows the inference that at least two widely separate areas are responsible for the inhibition involved in the rebound. Thus, although ablation of the cortex anterior to the cruciate sulcus almost always increased the initial response at the expense of the rebound, it did not always abolish inhibitory influences completely.

Since this remaining inhibitory element was ordinarily not modified by any section above the level of the anterior hypothalamus but could never be demonstrated in preparations with sections below its posterior limit (p. 269), the main site of the inhibition persistent after cortical ablation may safely be placed in this region. Such a localization is in agreement with the finding of inhibition on stimulation of the hypothalamus reported here, p. 267, and with the observations of Kabat, Magoun and Ranson (1935) for blood pressure.

The lowest center for excitatory effects found in these experiments was in the medulla at the level of the pons, and contributed much less to the reflexes studied than did the higher centers (fig. 6A, B and C).

As was pointed out above, the appearance of rebounds suggested a nice balance between central inhibition and excitation. Slight upsets of this balance could be discerned in modifications of the rebound, more extensive imbalance became evident in variations in total height of the responses, most apparent in the spontaneous variations in the extent of the reflexes outlined in the previous section.

One point in regard to the nature of the inhibitory influences studied is worth emphasizing. In no instance was evidence obtained that c.i.s. destroyed c.e.s. permanently. Indeed, if such were the case the phenomenon of excitatory rebound could not occur. Likewise stimulation of a cortical inhibitory area did not abolish the c.e.s. responsible for after-discharge, as in figure 10, but only reduced the height of contraction during the maintenance of the stimulus. Various explanations are possible depending upon the hypothesis accepted for the explanation of excitatory after-discharge. The simplest explanation satisfactory for either the chemical or reverberating chain theory is that the inhibition occurs at some synapse nearer the final motoneuron than the centers responsible for the after-discharge. Clearly none of the inhibitory areas studied, including peripheral afferents and central regions, was capable of damping "reverberating arcs" directly.

No explanation was apparent for the differences in the effects on the

two membranes occasionally encountered in stimulating either afferent nerves or central areas. The inconstancy of the differences in laterality merely served to emphasize the labile nature of the "centers" involved, and the complexity of their interrelation.

The exploration of the subcortical areas of the forebrain reported here was not carried out with the careful detail which characterized the work of Ranson and his collaborators (Ranson, Kabat and Magoun, 1935; Kabat, Magoun and Ranson, 1935; Kabat, Anson, Magoun and Ranson, 1935). The results, nevertheless, were in general agreement. The main discrepancy, namely, that excitation of sympathetic systems from regions rostral to the anterior commissure was obtained in the present investigation but not by Ranson and his co-workers, was in all probability due to the differences in the experimental conditions. Rioch and Brenner (1937) also noted dilatation of the pupils from stimulation of these areas in cats without neo-cortex. It was clear, however, that the chief sympathetic centers were located in the hypothalamus.

The ablation and stimulation experiments reported here indicate that the chief sympathetic inhibitory mechanisms are localized in the neocortex, probably mainly in the frontal poles (cf. Bard, 1934). The subcortical inhibitory mechanisms, though not negligible, were in no sense comparable to the excitatory mechanisms of the hypothalamus in effectiveness. The exact localization of these inhibitory "centers" was not accomplished by the experimental methods used, but the structures which came under consideration were: the region ventral to the putamen at the level of the rostral pole of the amygdala; the rostral, basal portion of the septum; and the anterior pole of the medial hypothalamus. Evidence that the cell bodies or dendrites of the neurones involved were located in these areas was as follows. The two areas first mentioned, defined by electrical stimulation, lay close to the periphery of the remaining brain in the decorticate cats. In several preparations with extensive acute ablations which did not involve the last mentioned area, a small, but definite inhibitory component remained in the sciatic-n.m. reflex; whereas no such inhibitory component could be demonstrated in any preparation with a lesion which included this area.

The experiments reported here clearly indicate that not only do the several sympathetic reflexes have discrete central representations but that any one reflex involves widely separated central areas. There is also evidence that certain hypothalamic mechanisms exert a coördinated effect on the sympathetic system as a whole (Bard, 1928). It should be pointed out, however, that the creation of the concept of an "emotional center" on the basis of such evidence is unjustifiable (Bard, 1934). The data available indicate that in animals, either normal or with lesions of the

brain, all the extant areas play a rôle not only in complex behavior patterns but also in discrete reflexes.

SUMMARY

The reflex responses of the nictitating membrane (n.m.) to various intensities and frequencies of stimulation were studied in urethanized cats with the forebrain either intact or sectioned at various levels.

The responses of intact animals were found to vary in total extent, in relative proportion of excitatory rebound, and in the duration of afterdischarge (fig. 3) irrespective of variations in the intensity, frequency, or duration of stimulation.

Evidence was presented that these variations were due to sudden and unexplained changes in the "excitability" or activity of the higher "centers" involved in the reflex (p. 271).

Stimulation of the cortex or of the basal ganglia was found either to inhibit (figs. 7A, 9, 10, 11) or to facilitate the reflex responses (figs. 4B, 5, 7B).

Inhibition prevented the effects of excitatory stimulation or its afterdischarge only during the period of inhibitory stimulation, i.e., c.i.s. did not cancel c.e.s. permanently (figs. 10, 11).

The results of both ablation (fig. 6) and stimulation experiments indicated that at least three widely separated areas may contribute to the excitatory component of the reflex responses of the n.m. These areas were located as follows:

a. In the medulla at the region of the pons.

b. At the level of the posterior hypothalamus.

c. In the cortex: principally that portion lying on both margins and in the depths of the cruciate sulcus (fig. 2).

At least two areas contributed inhibitory components: a, the cortical tissue surrounding the presylvian sulcus and extending laterally into the gyrus orbitalis (fig. 2); b, an area at the level of the anterior hypothalamus.

Various other areas stimulation of which produced facilitation or inhibition of the n.m. or of blood pressure but which ablation experiments showed to be unimportant in the reflex responses were described (fig. 2).

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A NEW PHYSIOLOGICAL VARIABLE ASSOCIATED WITH SENSIBLE AND INSENSIBLE PERSPIRATION

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It is a well known fact that one function of sweating is the maintenance of a constant body temperature in an environment where the loss of heat by radiation and convection is insufficient for the purpose. It has been shown (Winslow, Herrington and Gagge, 1936) that for environments where the operative temperature[1] lies above 31°C., the increase in heat loss by evaporation for nude subjects equals exactly the decrease in loss by radiation plus convection, as one moves toward higher operative temperatures. That there are experimental methods of determining the limits of efficiency of this process has been demonstrated (Gagge, 1936). It is the purpose of the present analysis to describe and discuss the physical processes involved in body temperature regulation by sensible perspiration and to demonstrate the fundamental interrelations involved between this perspiration and metabolism, storage, evaporation, skin temperature, dry bulb temperature, and relative humidity.

The rate of evaporation from the surface of a liquid is proportional to the difference between the vapor pressure in the liquid and the vapor pressure of the surrounding air. If we let e' represent the vapor pressure in the liquid and e the vapor pressure of the air, then the rate of evaporation, ξ, per unit area from the liquid surface will be

$$\xi = \mu(e' - e), \tag{1}$$

where μ is the constant of proportionality. Actually μ is a function of the air movement and of its direction of application in relation to the liquid surface. For purposes of the present analysis let us consider these factors as fixed and constant in magnitude. Equation (1) may be easily applied to the human body as e' is the saturation pressure at skin temperature, which we will write as the symbol $\epsilon(T_S)$, and e is the vapor pressure of the ambient air, which is, of course, the product of the relative humidity, rh (as a fraction), and the saturation vapor pressure at dry bulb tem-

[1] A measure devised by us which represents the properly weighted influence of air and wall temperature combined.

perature, $\epsilon(T_A)$. Thus, for the case of evaporation from skin, we may write (1)

$$\xi = \mu[\epsilon(T_S) - rh \times \epsilon(T_A)], \tag{2}$$

where the constants are so chosen that ξ is in kilogram-calories per hour per square meter per centimeter of Hg pressure. It must be noted here that ξ represents the evaporation from a unit area of a surface completely covered by liquid at skin temperature. If E/A or E' is the total evaporation per unit area from the human body surface, the ratio of E'/ξ or w will represent the fraction of this unit body surface covered by liquid perspiration. Thus, w is a measure of the degree of wettedness involved in the process of evaporation represented by E and may be expressed as a percentage.

Combining the above equations, we have the general relation

$$\begin{aligned} w &= E'/\xi \\ &= E'/\mu[\epsilon(T_S) - rh \times \epsilon(T_A)], \end{aligned} \tag{3}$$

or

$$(w\mu) = E'/[\epsilon(T_S) - rh \times \epsilon(T_A)]. \tag{4}$$

As we saw above, w represents that fraction of the body surface which—if completely wetted—would produce the observed effect. The term, μ, constant only for a given air movement and direction of air movement, represents the coefficient of heat transmission by evaporation in kilogram-calories per square meter per hour per centimeter of Hg of vapor pressure difference. Its actual magnitude is not necessarily known. However, the product $(w\mu)$ represents a fundamental property of the skin surface and is a *direct* measure of the extent of the liquid surface available for evaporation. In the process of evaporation by the human body, when placed in an environment of varying air temperature and humidity, there is a maximum value for the product $(w\mu)$ where further excretion of sweat is no longer efficient. Beyond this point the sweat runs off and evaporation takes place at points other than on the surface of the body. On the other hand, there is also a minimum value for $(w\mu)$. This minimum value describes the cases where only insensible perspiration exists and measures the permeability of the skin to moisture from the inner body regions. With fixed conditions of air movement the maximum value of $(w\mu)$ describes the skin surface at its maximum degree of perspiration coverage. The minimum value describes the normal dampness of the skin. The purpose of the analysis that is to follow is to evaluate $(w\mu)$ at both extremes of its range of variability and fix these values as definite biological constants. Finally, a correction for air movement will be introduced.

Maximal values for $(w\mu)$. In a previous publication (Gagge, 1936), it has been shown that the direct measure of evaporation fails when

perspiration is secreted in such large amounts that evaporation takes place from other surfaces than the body surface. In figure 1b of that paper, deviations from linearity were noted at the extremes of both the hot region and the cold region, when the algebraic sum of measured metabolism, measured storage (from rectal temperature changes) and measured evaporation were plotted against radiation intensity. For those points toward the colder regions the deviation was ascribed to an error in the value of storage as derived from the changes in rectal temperature. Toward the warmer conditions the deviation was shown to be an error in the measurement of total evaporation. The actual deviation from linearity is a direct measure of this error, since for this region storage was probably correct as measured by changes in rectal temperature. The actual effective degree of wettedness may be calculated using the true total evaporation for E in equation (3).

The true value for E may be computed from the fundamental partition equation,

$$E = M + S + R + C, \tag{5}$$

where M is the metabolism, S the storage, as measured by changes in rectal temperature, R the total radiation exchange, and C the total convection exchange. Substituting (5), equation (4) therefore becomes,

$$(w\mu) = (1/A_D)(M + S + R + C)/[\epsilon(T_S) - rh \times \epsilon(T_A)] \tag{6},$$

where A_D is the DuBois total body area.

Even when the evaporation determined by weight loss is larger than the evaporation loss effective in body-cooling, it is nevertheless true that the actual degree of wettedness does not vary. The quantity of moisture on the skin surface may very well increase under such conditions, but the surface of moisture exposed to the environment remains the same. It is obvious that no value of w can ever be greater than unity, or 100 per cent, i.e., each unit area of skin surface cannot expose a liquid surface greater than its own actual area. The maximum value of the physical variable $(w\mu)$ may therefore be calculated accurately from those points on the linearity curve where deviations take place, provided the true evaporation is used instead of that actually measured by weight loss.

In table 1 are presented calculations and partitions of the actual experiments which lie above the linear region toward the warmer ends of the curves in figure 1b of the earlier paper (Gagge, 1936). There are four experiments for subject I and five for subject II. These partitions are carried out by using the direct measurements of metabolism, storage, radiation, and convection. The true evaporation is found by difference and is compared with the observed evaporation. The column headed by

($w\mu$) has been calculated from equation (6) above. For subject I the mean value of this constant is 29.5 kilogram-calories per square meter per hour per centimeter of Hg pressure. For subject II its mean value is 32.0. If we exclude experiment 109-A which had an extremely high value, the mean for this subject is 30.3. Although the degree of variability of this constant for subject II is greater than for subject I, it is significant that between these two subjects of quite dissimilar characteristics,[2] the calculated value for ($w\mu$), excluding experiment 109-A, is essentially constant with a mean value of 29.9 kilogram-calories per square meter per hour per centimeter of Hg pressure difference. This constant, however, is sig-

TABLE 1

The probable value of ($w\mu$) at its maximum value

EXPERIMENT	T_W	T_A	RH	T_S	$\epsilon(T_S)$-rh$\epsilon(T_A)$ or ξ/μ	A_D	M	S	R	C	TRUE E	OBSERVED E	($w\mu$)
	°C.	°C.	per cent	°C.	cm. of Hg	sq.m.	kgm.-cal. / hr.						kgm.-cal. / m²-hr.-cm. of Hg
I-106-A	52.6	29.7	44	35.6	2.96	2.13	98	0	147	−56	−189	−228	30.1
I-106-B	52.7	29.7	43	35.6	3.00		95	−8	149	−56	−188	−214	29.5
I-107-A	52.3	29.4	43	35.3	2.96		100	−14	150	−56	−180	−211	28.5
I-107-B	52.5	29.1	46	35.3	2.87		84	0	152	−59	−177	−212	29.1
Mean value													29.5
II-109-A	53.7	30.0	47	35.5	2.85	1.49	88	−11	123	−45	−165	−169	38.8
II-109-B	53.3	30.3	43	35.9	3.02		75	0	112	−45	−142	−170	31.6
II-110-A	53.3	30.0	42	34.9	2.88		82	−11	117	−40	−148	−155	34.5
II-110-B	52.6	29.1	44	35.6	3.00		73	− 5	109	−52	−125	−156	27.5
II-111-A	52.4	28.9	44	35.7	3.01		82	0	110	−55	−137	−157	30.1
II-111-B	51.7	29.1	44	35.8	3.05		73	0	105	−54	−124	−164	27.3
Mean value*													30.3

* Excluding II-109-A.

nificant only for a turbulent air movement of 17 feet per minute, the particular condition of our experiments. For this condition, this value, therefore, corresponds to the maximum possible degree of wettedness of the moist surfaces of the body.

In table 1 no correction has been made for evaporation from the respiratory passages. For the experiments in table 1 the mean respiratory evaporation was 8 kilogram-calories per hour for subject I and 7 kilogram-calories per hour for subject II. Subtracting these values from the true

[2] One a pronounced pyknic, the other a leptosomic subject.

TABLE 2

Experiments covering the complete range of ($w\mu$) values

GROUP	T_W	T_A	RH	T_O	T_S	$\xi\mu$	A_D	LUNG VENTILATION	TOTAL E	RESPIRED E	SKIN E	TOTAL ($w\mu$)	SKIN ($w\mu$)
	°C.	°C.	per cent	°C.	°C.	cm. of Hg	sq.m.	lit./min.	kgm.-cal./hr.			kgm.-cal./m²-hr.-cm. of Hg	
I-1	52.4	29.4	44	41.0	35.1	2.982	2.13	7.7	192	8	184	30.2	28.9
2	56.3	24.2	44	40.5	35.2	3.199		7.8	184	9	175	24.2	22.5
3	46.4	29.6	41	38.0	34.4	2.812		7.9	160	8	152	26.7	25.4
4	49.7	24.5	42	37.0	34.7	3.240		7.2	151	8	143	21.9	20.7
5	54.8	17.6	44	36.5	34.2	3.406		8.9	137	11	126	19.0	17.4
6	36.6	35.4	40	36.0	35.0	2.472		7.1	99	6	93	18.8	17.6
7	53.3	16.4	42	35.5	33.9	3.354		8.4	101	11	90	14.1	12.6
8	40.6	29.0	46	35.0	34.8	2.833		7.5	98	8	90	16.3	14.9
9	35.8	33.6	42	34.5	34.7	2.595		12.6	89	11	78	16.1	14.1
10	34.6	33.3	45	34.0	34.2	2.353		7.7	70	7	63	14.0	12.6
11	35.8	31.5	44	34.0	34.6	2.539		12.4	67	11	56	12.4	10.5
12	34.8	31.3	46	33.5	34.5	2.518		7.8	61	8	53	9.6	9.9
13	49.1	16.0	46	32.0	34.8	3.576		7.8	44	10	34	5.8	4.5
14	41.3	23.2	40	32.0	33.8	3.096		7.0	41	8	33	6.2	5.0
15	35.8	28.7	50	32.0	34.5	2.585		11.3	41	11	30	7.6	5.4
16	34.3	27.0	42	31.0	34.0	2.817		15.2	36	17	19	6.0	3.1
17	48.8	12.6	46	30.5	32.5	3.204		7.6	55	10	45	8.1	6.6
18	38.9	21.6	44	29.5	32.8	2.864		7.5	33	9	24	5.4	3.9
19	38.6	18.1	40	28.5	33.6	3.323		7.0	32	9	23	4.5	3.3
20	33.3	23.2	40	28.5	32.2	2.745		8.7	34	10	24	5.8	4.1
21	28.6	27.6	58	28.5	33.4	2.214		7.2	34	6	28	7.2	6.0
22	30.0	24.7	52	28.0	32.8	2.482		8.9	37	9	28	7.0	5.2
23	33.8	19.5	42	26.5	31.9	2.890		7.3	37	9	28	6.0	4.7
24	39.4	12.6	50	26.0	30.7	2.724		6.5	45	8	37	7.8	6.4
25	44.2	6.8	30	25.5	29.2	2.853		6.5	28	9	19	4.7	3.1
26	33.0	17.2	42	25.0	31.2	2.761		8.3	29	11	28	4.8	3.1
27	31.8	16.1	50	24.0	30.3	2.590		7.8	36	10	29	6.6	5.2
28	28.4	19.0	50	24.0	31.0	2.565		7.8	33	9	24	6.0	4.5
29	24.1	22.6	65	23.5	30.9	2.033		8.6	22	9	13	5.0	2.9
30	31.4	13.7	42	23.0	29.7	2.575		7.0	29	9	20	5.2	3.7
31	31.5	12.0	48	22.0	29.0	2.477		6.5	30	8	22	6.0	4.5
32	33.3	8.5	40	21.0	28.0	2.219		7.7	25	11	14	5.2	2.9
33	29.1	12.8	47	21.0	28.8	2.461		8.1	27	11	16	5.2	3.3
34	19.0	17.1	60	18.5	28.4	1.992		8.5	22	10	12	5.2	2.9
35	22.2	13.1	46	18.0	28.2	2.358		7.0	23	9	14	4.7	2.7
II-1	52.8	29.6	44	40.5	35.6	2.957	1.49	7.1	133	7	126	30.2	28.5
2	59.0	24.0	38	39.5	35.7	3.498		6.0	140	7	133	26.9	25.6
3	47.3	29.6	41	38.0	34.9	2.946		6.2	106	7	99	24.2	22.5
4	49.7	24.1	41	36.0	35.0	3.297		5.4	79	6	73	16.1	14.9
5	36.6	35.5	39	36.0	35.1	3.029		5.8	76	5	71	16.9	15.7
6	54.3	17.0	43	34.5	34.6	3.447		6.5	80	8	72	15.5	14.0

TABLE 2—*Concluded*

GROUP	T_W	T_A	RH	T_O	T_S	ξ/μ	A_D	LUNG VENTILATION	TOTAL E	RESPIRED E	SKIN E	TOTAL ($w\mu$)	SKIN ($w\mu$)
	°C.	°C.	per cent	°C.	°C.	cm. of Hg	sq.m.	lit./min.	kgm.-cal./hr.			kgm.-cal./m²-hr.-cm. of Hg	
7	40.7	27.7	48	34.5	34.3	2.652		6.9	63	7	56	15.9	14.1
8	35.2	33.2	45	34.0	34.9	2.993		12.0	67	11	56	15.1	12.6
9	36.1	31.4	46	33.5	34.9	2.606		12.3	67	11	56	17.2	14.3
10	34.5	31.2	44	33.0	34.8	2.719		10.0	60	9	51	14.7	12.6
11	42.9	23.3	50	32.0	34.3	3.003		5.8	58	7	51	13.0	9.6
12	49.4	16.6	38	31.5	34.8	3.669		14.6	42	18	26	7.8	4.8
13	35.8	28.3	50	31.5	34.4	2.637		8.2	33	8	25	8.3	6.4
14	48.1	16.3	42	31.0	35.1	3.633		9.7	51	12	39	9.5	7.2
15	50.5	14.0	36	30.5	34.2	3.648		6.0	30	8	22	5.4	4.1
16	34.6	28.0	40	30.5	34.6	2.993		11.9	39	13	26	8.7	5.8
17	41.8	17.8	44	29.0	32.6	3.044		6.8	32	8	24	7.0	5.2
18	36.9	22.1	42	28.5	33.4	2.993		6.0	24	7	17	5.4	3.9
19	28.3	27.5	67	28.0	32.8	2.125		6.6	21	6	15	6.6	4.7
20	33.6	23.0	42	28.0	32.8	2.843		7.6	30	9	21	7.2	5.0
21	34.4	19.3	43	26.1	32.1	2.859		6.4	16	8	8	3.7	1.7
22	44.4	7.7	28	24.5	29.6	2.802		6.5	23	9	14	5.4	3.3
23	38.7	12.3	48	24.5	31.1	2.869		6.0	20	8	12	4.7	2.7
24	33.0	17.5	49	24.5	30.9	2.632		7.3	25	9	16	6.4	4.1
25	31.8	16.8	47	24.0	30.7	2.611		6.4	29	8	21	7.4	5.4
26	25.1	22.7	60	24.0	31.3	2.136		8.5	25	9	16	7.9	5.0
27	27.1	18.7	40	33.0	30.4	2.626		5.4	20	7	13	5.0	3.3
28	23.3	22.5	67	23.0	30.7	1.894		7.0	22	7	15	7.8	5.2
29	32.7	13.6	42	22.0	30.4	2.776		6.4	21	8	13	5.0	3.1
30	31.4	14.0	46	22.0	29.9	2.626		6.2	22	8	14	5.6	3.5
31	34.7	8.7	34	20.5	28.5	2.590		6.5	21	9	12	5.4	3.1
32	31.1	11.6	40	20.5	29.7	2.663		6.0	18	8	10	4.5	2.5
33	29.1	13.0	55	20.5	29.6	2.466		5.6	20	7	13	5.4	3.5
34	22.5	13.2	45	18.0	28.5	2.343		6.0	17	8	99	4.8	2.5
35	19.0	17.1	51	18.0	29.6	2.327		5.6	17	7	10	4.8	2.9

evaporation, we find for the mean value of ($w\mu$) for subject I, 28.1 kilogram-calories per square meter per hour per centimeter of Hg pressure; for subject II, 28.7. The mean value for both subjects is 28.5 kilogram-calories per square meter per hour per centimeter of Hg pressure. This value represents the maximum possible degree of wettedness of the skin surface alone.

In summing up, we note that the value of ($w\mu$) regardless of individual interpretation of the factors w and μ themselves cannot rise above 29.9 for the total moist surfaces of the body or above 28.5 for the skin surfaces alone. This value applies only for the turbulent air movement of 17

feet per minute, or 8.65 cm. per second. In a later paragraph it will be shown how this value may be altered at other rates of air movement.

Minimal values for ($w\mu$). In a previous publication (Winslow, Herrington and Gagge, 1937), we described 35 groups of experiments for subjects I and II extending over a considerable range of air and wall temperatures and a range of operative temperatures from 17°C. to 41°C. It was shown that, above and below the critical temperature of 31°C., the body acted in two distinct ways. Above, the regulation of body temperature was accomplished by evaporation principally; below, the balance was maintained by storage. Above 31°C., the total evaporation from the body was found

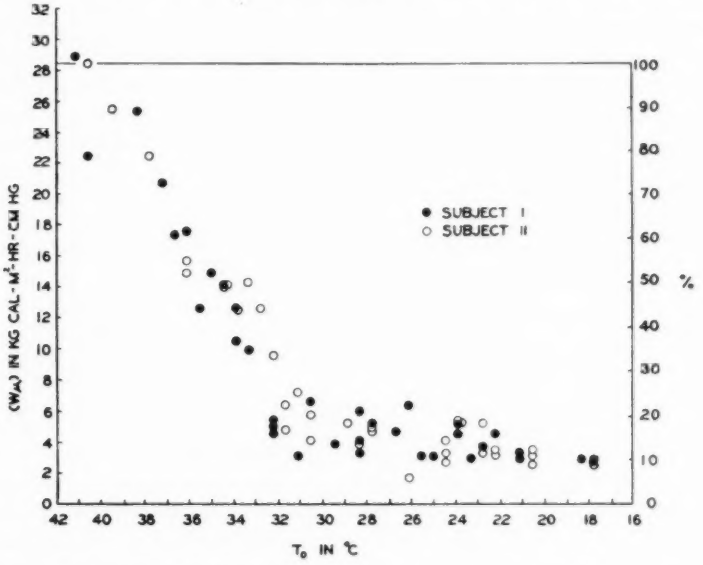

Fig. 1. Relation of the degree of wettedness of skin surface ($w\mu$) to operative temperature (T_o).

to be linear with the operative temperature; below 31°C. the evaporation was insensible and showed a slight decrease toward the lower values of T_o. In table 2 are presented the values of ($w\mu$) for the 35 groups for both subject I and subject II. The value of ($w\mu$) is calculated by equation (6) for the total evaporation as well as for the skin surface by itself.

The values for ($w\mu$) for the skin surface only have been plotted against T_o in figure 1. The points for subject I are in closed circles, while those for subject II are in open circles. Above 31°C., there is a continual, although slightly irregular, rise in ($w\mu$) toward its theoretical maximum value at 28.5. Below 31°C. it will be noted that ($w\mu$) is relatively con-

stant. There seems to be a very definite minimum below which, with one possible exception, the values do not fall. This value for $(w\mu)$ is approximately 2.9. In this zone of T_o below 31°C., we have the mean values for $(w\mu)$ shown in table 3 for the zone of insensible perspiration. Therefore, in the region below 31°C. the average minimal value of the degree of moisture as indicated by $(w\mu)$ for the whole body is 5.96, while the value for the skin alone is 4.05. It is of interest to note that the degree of moisture contributed by the respiratory passages is roughly 1.91 in terms of $(w\mu)$.

Of special interest is the fact that in the zone of insensible perspiration (below 31°C.) the degree of moisture $(w\mu)$ of the skin surface alone, as well as that of the total body, remains essentially constant and the decrease in total evaporation noted in our earlier papers is due to changes in the evaporating power of the environment only, not to changes in the physiological properties of the skin itself.

Computation of values of $(w\mu)$ *for the case of still air.* It has been shown

TABLE 3

SUBJECT	MEAN $(w\mu)$ FOR TOTAL MOISTURE SURFACES	MEAN $(w\mu)$ FOR SKIN MOISTURE ONLY
I	5.83	4.10
II	6.08	3.99
Mean	5.96	4.05

by Coffey and Horne, and by Carrier (American Society of Heating and Ventilating Engineers, 1936) that for a transverse flow of air across a vertical tubular moisture surface the total heat transmitted by evaporation is proportional to $(1 + v/121)$ where v, the air velocity is given in centimeters per second, and that for a parallel flow of air on a horizontal surface the heat transmitted by evaporation is proportional to $(1 + v/117)$. For our case of turbulent air motion, where the air movement may satisfy either one or both these situations, let us assume the heat lost by evaporation is $(1 + v/119)$, which is quite reasonable in view of the widely different situations in which the values of 117 and 121 were determined. Hence, if μ_0 is the value of the evaporational constant for still air, then equation (1) becomes

$$\xi = \mu_0(1 + v/119)\ (e' - e), \tag{7}$$

whence our calculated value for $(w\mu)$ becomes

$$(w\mu_0)\ (1 + v/119). \tag{8}$$

In table 4 are presented the extrapolated values of $(w\mu)$ for still air. These values represent fundamental physiological constants for the human body.

It might be mentioned at this point that, for purposes of analysis only may one use (8) to calculate values of the physiological constant $(w\mu)$ for higher air movements. There has been no evidence presented so far to indicate that the maximum and minimum values of $(w\mu)$ are functions of the air movement alone. There is a reasonable possibility that physiological complications may enter. It might be mentioned, too, that an extrapolation by use of (8) would apply only for the case of turbulent air motion.

The practical application of $(w\mu)$. In table 4 the still-air value for $(w\mu)$ at its maximum was found to be 26.6 for the skin surface itself. We pointed out in the opening paragraphs that in general w is a fraction (or a percentage) which varies between zero and unity (0–100 per cent) and, if μ were known accurately, it would represent the fraction of skin surface that is actually wet. However, the maximum value of w occurs at the same

TABLE 4

RANGE	$(w\mu)$ FOR TOTAL BODY	$(w\mu)$ FOR SKIN SURFACE
Maximum value	27.9	26.6
Minimal value	5.42	3.81
Minimum value	3.64	2.04

time that $(w\mu)$ is at its maximum value. If, at this particular condition, we assumed the total skin surface to be 100 per cent wet, one would derive a value of 26.6 for μ. It is important to note that μ cannot have a value in any of our experiments less than 26.6. If the maximum value of w were 80 per cent, then μ would equal 33.2; or if 60 per cent, 44.4; and so on. In the experiments mentioned above by Coffey and Horne, and Carrier, for the case of a parallel flow and a horizontal surface, a value of 51.0 was found for μ. For the case of a transverse flow and a vertical surface, μ was 106. However, the experiments cited were designed neither for the particular case of turbulent air flow nor for an irregular shaped contour such as that of a human body. It is impossible at the present stage of our work to assign a definite value to μ and thus convert w to an actual fraction of the body area. However, as will be shown later, this restriction does not handicap us in the application of the formula for $(w\mu)$ above to various physiological problems.

In actual application to experiments the physiological variable $(w\mu)$ as it stands, has no explicit meaning in the absence of a definite value for

μ. However, as pointed out above, we know that w is at its maximum when the product $(w\mu)$ has reached its maximum limit. If we interpreted w as the fraction of the total possible wetted area of the body; then for the maximum case w would be 100 per cent. w would now be described as the percentage of (maximum) wettedness of the skin surface. For the points in figure 1 the whole range of $(w\mu)$ from 0 to 28.5 may be divided into 100 parts. The percentage for the points in that curve are indicated by the right hand axis of the chart. We see the maximum wettedness falls at about 100 per cent and extends down to the minimum value of approximately 10 per cent.

Discussion. A word might be said about the place of the present method for the measurement of the extent of sweat secretion in relation to others currently used in the study of sweat gland activity.

The actual observation of sweat secretion and its intimate processes is often done microscopically by the Jürgensen oil drop method (1924). The area of secretion, the number of secreted droplets and their approximate size may be observed in the field of vision of the microscope, and a measure derived which gives the sweat volume per unit area of field. By such sampling processes these measurements could be used to estimate w, the fractional area of wettedness of the body surface. In such a laborious experiment the perturbing effect of the source of illumination and the method of observation may complicate considerably the gross phenomena under observation. The $(w\mu)$ method for the measurement of the extent of sweat secretion, as a macroscopic procedure, is relatively simpler, and probably yields a more reliable measure of the secretory function, insofar as we consider it, in direct relation to the general mechanisms by which thermal homeostasis is maintained. The Jürgensen method, on the other hand, has no good substitute in situations where attention is centered on local activity and its relation to changes within the body system, rather than in the body-environment complex.

A second method of measuring sweat secretion may be derived from observation of changes in its saline content. Evaporation of water from a surface secretion of a given initial concentration in which the secretion is continuous should result in a gradual increase in the concentration of the evaporating fluid. The particular virtue of the "saline" method lies in the provision of a chemical approach to the physiological origin of the secretion itself. Variable factors in the process and technical difficulties inherent in the sampling method do not recommend this procedure as a measure of the amount or extent of secretion, although the increase in concentration may be a measure of the total evaporation from the body surface. This may be directly measured, however, by the time rate of weight loss.

In the present method no correction has been made for the effect on

vapor pressure related to the saline content of the secreted sweat. In general, observers agree that sweat is hypotonic in relation to blood. The vapor pressure of blood at body temperature differs by approximately two millimeters of Hg from the vapor pressure of water at the same temperature. An effect related to a vapor pressure change of this order is believed to be quantitatively unimportant for the present stage of the analysis.

SUMMARY

A new physiological variable ($w\mu$) is defined, which describes the extent of moisture surface present on the skin surface of the body. For a turbulent air motion of 8.65 cm. per second, the maximum value of ($w\mu$) for the body skin surface is found to be 28.5 kilogram-calories per square meter per centimeter of Hg vapor pressure; its minimum value, 2.9. For operative temperatures below 31°C., ($w\mu$) remains relatively constant at about a minimal value of 3.81. These values represent definite physiological constants, regardless of the interpretation and magnitudes applied to w and μ, individually. A useful interpretation of w and μ is one such that w is unity, when the extent of moisture surface is at its greatest, and when μ assumes the value of 28.5.

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PHYSIOLOGICAL REACTIONS OF THE HUMAN BODY TO VARIOUS ATMOSPHERIC HUMIDITIES

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Plan of studies. As in the earlier studies from this laboratory[1] our observations were made on unclothed male subjects in a semi-reclining position, placed in a copper booth, so arranged that air temperature and radiation from the copper walls could be independently varied. In each experiment the heat interchange between the body and the environment has been analyzed into its five factors—metabolism, radiation, convection, evaporation, and storage—by methods described in detail in the papers cited.

In our earlier work, we maintained, in general, a relative humidity of 40 to 50 per cent of saturation while varying air temperature, radiation and air movement. In the present studies we have provided a uniform turbulent air movement of approximately 17 linear feet per minute (8–9 cm./sec.) and no artificial radiation was employed, so that air and wall temperatures assumed a common value: air (and wall) temperature and relative humidity were the variable factors.

Three subjects were used for the present experiments, whose physical characteristics were as follows:

SUBJECT	HEIGHT METERS	WEIGHT KILOGRAMS	DUBOIS LINEAR AREA SQUARE METERS	EFFECTIVE RADIATION AREA, SQUARE METERS[2]	CONSTANT FOR HEAT LOSS BY CONVECTION AND RADIATION FOR AIR VELOCITY 17 FEET[2]
I	1.70	105.0	2.13	1.58	18.0
IV	1.65	50.8	1.53	1.02	12.2
VII	1.88	79.0	1.92	1.63	18.5

General results. The present study is based on some 300 different experiments and to save space the results are presented in table 1 in the form

[1] Winslow, Herrington and Gagge, 1936a and b, and 1937; Gagge, 1936; Herrington, Winslow and Gagge, 1937.

[2] The methods of computing the factors are described in our earlier papers.

of group averages, each group including about six different experiments in which air temperature and atmospheric humidity were approximately identical. The headings of the columns and the method of computing the various factors are explained in a footnote to the table and in the text below.

It will be noted that air temperatures varied from 16.6°C. to 37.9°C., and relative humidities from 14 to 80 per cent of saturation. Heat losses by radiation and by convection were, throughout, of almost exactly equal magnitude and, of course, both types of heat loss increased with decreasing environmental temperature according to principles whose oper-

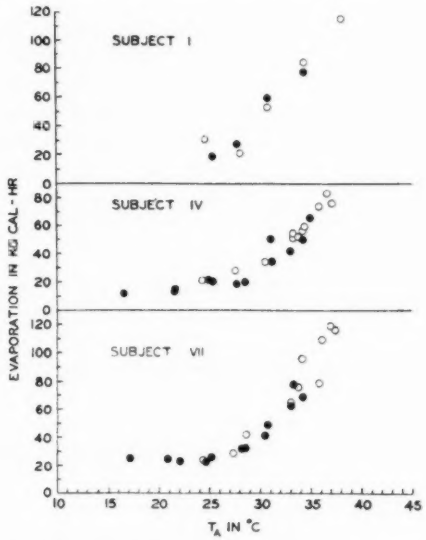

Fig. 1. Evaporative heat loss in relation to air temperatures. Solid circles, high humidity. Open circles, low humidity.

ation was made clear in our earlier studies. Heat loss by evaporation varied widely in a fashion to be discussed below.

Evaporative heat loss in relation to atmospheric humidity. In order to elucidate this point, we have plotted in figure 1 the data for evaporative heat loss for each of the three subjects in relation to atmospheric temperature. All groups of experiments with a relative humidity below 40 per cent are plotted as open circles and all groups with relative humidities higher than 40 per cent as closed circles.

These graphs, first of all, bring out the major relations between evaporative heat loss and atmospheric temperature which we have discussed in

TABLE 1*

Group means of experimental data (Subjects I, IV, VII)

GROUP	NUMBER OF EXPERIMENT	T_A	RH	T_S	T_B	M	E	R	C	TRUE S	CONDUCTANCE	LUNG VENT.	RESP. EVAP.	SKIN EVAP.	EVAP. POWER	PER CENT OF WETTEDNESS
		°C.	per cent	°C.	°C.	kgm.-cal. hr.	kgm.-cal. hr.	kgm.-cal. hr.	kgm.-cal. hr.	kgm.-cal. hr.	kgm.-cal. m.²/hr./°C.	liters min.	kgm.-cal. hr.	kgm.-cal. hr.	kgm.-cal. m.²/hr.	per cent
I-1	6	24.4	35	31.8	36.7	93	−31	−62	−63	63	16.4	7.8	9	22	76	13
2	4	27.8	26	32.9	37.3	88	−21	−43	−46	22	11.9	8.0	10	11	84	6
3	6	30.6	22	33.7	36.6	87	−54	−26	−28	21	17.8	8.4	10	44	92	22
4	4	34.3	17	34.5	37.0	89	−85	−1	−2	−1	16.7	7.8	9	76	96	37
5	4	37.9	17	35.4	38.4	85	−116	22	23	−14	11.2	11.6	14	102	100	46
6	6	25.2	75	32.4	36.6	94	−19	−61	−65	51	16.7	7.7	7	12	52	11
7	6	27.6	75	32.8	36.9	99	−27	−44	−48	20	13.9	7.3	5	22	45	23
8	4	30.6	60	34.9	36.8	103	−60	−36	−38	31	32.8	8.5	7	53	63	40
9	6	34.3	78	35.6	37.0	99	−78	−11	−13	3	34.4	7.5	3	75	35	100
IV-1	6	24.3	32	31.0	37.3	62	−22	−40	−45	45	11.8	4.7	6	16	74	14
2	6	27.5	27	32.5	37.2	58	−29	−28	−32	31	12.1	5.2	6	23	81	19
3	6	30.4	22	33.4	36.8	62	−35	−16	−19	8	13.0	5.5	7	28	88	21
4	6	33.2	20	35.4	36.9	66	−55	−13	−15	17	35.1	5.5	7	48	101	31
5	6	33.8	27	35.4	36.7	61	−53	−10	−11	13	25.4	5.5	6	47	93	33
6	6	34.2	38	35.2	36.6	74	−57	−6	−7	−4	32.6	5.5	5	52	77	44
7	6	34.4	19	34.3	37.2	57	−60	1	1	1	12.8	5.2	6	54	94	38
8	6	35.8	29	35.4	37.0	65	−75	3	3	4	27.2	5.7	6	69	94	48
9	6	36.5	25	35.7	36.9	64	−84	5	5	10	39.1	5.0	5	79	90	57
10	6	37.0	14	34.6	37.4	68	−75	14	15	−22	10.1	7.0	9	66	97	45
11	4	16.6	64	29.5	36.0	65	−12	−71	−89	107	16.9	5.8	7	5	63	5
12	6	21.6	44	31.9	36.6	61	−15	−59	−71	84	19.4	5.9	7	8	74	7
13	6	21.6	76	31.7	36.6	57	−14	−57	−69	83	18.0	5.5	5	9	57	10
14	6	24.9	48	33.1	36.7	70	−20	−47	−56	53	21.6	5.5	6	14	75	12
15	8	25.3	72	31.8	36.9	68	−19	−38	−42	31	12.4	4.5	4	15	50	20
16	6	27.7	75	33.5	36.9	67	−17	−33	−38	21	16.6	5.0	4	13	55	15
17	6	28.6	52	34.6	36.7	63	−21	−35	−41	34	28.2	5.5	5	16	73	14
18	6	31.0	63	34.7	36.8	72	−51	−21	−24	24	29.7	5.0	4	47	58	53
19	6	31.1	45	35.6	36.9	63	−35	−26	−30	28	42.2	5.1	5	30	79	25
20	6	33.0	52	35.1	36.6	67	−42	−12	−14	1	28.8	5.5	5	37	71	34
21	6	34.9	42	33.9	37.1	63	−66	6	7	−10	10.8	5.0	4	62	59	69
22	6	34.1	80	35.1	36.5	67	−51	−6	−6	−14	31.0	5.0	2	49	32	100
VII-1	6	24.3	32	32.2	37.2	104	−26	−71	−75	68	17.8	10.6	12	14	81	8
2	6	27.8	26	32.8	37.5	92	−29	−45	−47	29	13.3	7.8	9	20	87	12
3	6	30.6	22	34.5	37.2	102	−42	−36	−37	13	22.0	10.6	13	29	97	16

VII-1	6	24.8	32	32.2	37.2	101	−26	−71	−75	68	17.8	10.6	12	14	81	8
2	6	27.8	26	32.8	37.5	92	−29	−45	−47	29	13.3	7.8	9	20	87	12
3	6	30.6	22	34.5	37.2	102	−42	−36	−37	13	22.0	10.6	13	29	97	16
4	6	33.1	29	35.6	37.1	84	−65	−23	−25	29	37.8	9.3	11	54	99	28
5	6	33.8	36	35.8	37.1	97	−76	−19	−21	19	49.5	10.3	10	66	86	40
6	6	34.1	17	35.3	37.6	105	−96	−11	−11	13	26.3	10.5	13	83	104	42
7	6	35.8	22	35.9	37.3	96	−109	−1	−1	15	40.0	10.0	12	97	100	51
8	6	35.8	35	34.2	37.2	97	−79	15	17	−50	7.9	10.6	10	69	70	51
9	6	36.7	26	36.1	37.2	95	−119	5	6	13	50.5	10.3	11	108	93	60
10	6	37.4	16	35.8	37.8	86	−116	15	17	−2	19.6	14.2	17	99	103	50
11	6	17.1	70	29.7	36.7	96	−25	−115	−127	171	19.8	9.3	10	15	57	14
12	6	20.1	55	32.1	36.7	92	−25	−102	−113	148	26.8	11.0	10	15	72	11
13	6	22.1	78	32.6	36.8	91	−23	−96	−106	134	27.8	10.9	10	13	62	11
14	6	24.4	75	31.8	37.1	99	−24	−67	−70	62	15.9	7.5	7	17	51	17
15	6	25.2	48	33.2	36.5	82	−26	−73	−80	97	24.3	9.0	10	16	77	14
16	6	28.1	70	33.2	37.2	94	−32	−46	−48	32	16.2	8.1	6	26	52	26
17	6	28.6	52	35.0	36.7	85	−32	−59	−65	71	47.1	9.3	8	24	77	16
18	6	30.4	65	35.6	37.4	104	−41	−47	−48	32	38.7	9.0	7	34	63	28
19	4	30.7	47	35.9	37.1	91	−49	−47	−52	57	63.2	10.0	10	39	90	23
20	6	33.1	70	35.8	37.1	95	−63	−25	−28	21	47.4	9.3	5	58	48	63
21	6	33.6	45	35.8	37.0	98	−78	−21	−23	24	54.4	9.9	9	69	48	75
22	6	34.2	77	36.3	36.8	105	−69	−19	−20	3	101.3	7.5	2	67	35	100

* The first column gives the subject and the experimental group.
The dry bulb temperature (T_A) and the relative humidity (as a per cent) appear in the next two columns.
The skin temperature (T_S) is a mean for the whole body and represents a weighted mean of 15 segmental observations.
The body temperature (T_B) is measured rectally.
The metabolism (M) is measured by the rate of oxygen consumption.
The total evaporative loss (E) is derived from the rate of weight loss.
The radiation loss (R) and the convection loss (C) have been calculated from the usual formulae using the constants indicated in a previous table.
The true storage (S) has been found by difference.
The conductance (*Cond.*) represents the mean heat flux through the skin surface per degree of gradient fall to the skin temperature. It is calculated from the equation;

$$\frac{M + S}{A\,(T_B - T_S)} \text{ or } \frac{E + R + C}{A\,(T_B - T_S)},$$

where A is the DuBois or total area of the body.
The heat lost by evaporation in the respiratory tracts has been calculated from the lung ventilation assuming that the exhaled air is saturated and at body temperature.
The "skin evaporation" represents that evaporation taking place on the skin surface alone and is measured by the difference between the total evaporation and the respired evaporation.
The evaporating power of the air is proportional to the heat lost to the environment by a unit area of exposed liquid surface at skin temperature.
The "per cent of wettedness" represents the ratio of the skin evaporation per unit area to the evaporating power. It measures the fraction of the maximum possible sweat secreting surfaces that are actually stimulated.

For conditions I-9, IV-22, and VII-22, the measured total evaporation per hour was 93, 61, and 84 kilogram-calories per hour, respectively. These values would, however, have resulted in a per cent of wettedness over 100 per cent. The values in the table for E, namely, 78, 51, and 69, have been calculated such that the per cent of wettedness is exactly 100 per cent. These experiments represent situations in which the total surface water loss is not a measure of the effective evaporation.

our earlier communications. Below a certain critical point (about 30°C. air temperature)[3] evaporative heat loss is at almost a minimum level (15–25 kilogram-calories per hour) which corresponds closely to evaporation from the normal skin with no active secretion of sweat. Above this point evaporation rises rapidly with further increase of operative temperature.

The second fact brought out by figure 1 is that the relative humidity of the atmosphere, within the limits studied, seems to have no appreciable influence upon the magnitude of evaporative heat loss so long as physical conditions permit an evaporative balance. The open and closed circles in the graphs show no divergence, both alike exhibiting the same relationship to atmospheric temperature.

Physiological control of sweat secretion. It is, of course, obvious that this identical evaporative heat loss, at a given air temperature but with widely varying humidities, calls for a physiological explanation. If the surface of the body maintains the same physiological characteristics, there would necessarily be less evaporation with high relative humidity. The rate of evaporation from a unit surface of water is proportional to the difference between the vapor pressure of the liquid and the vapor pressure of the surrounding air and to the degree of air movement. The constancy of evaporative heat loss with varying relative humidity must be maintained, therefore, by changes in sweat secretion.

In a previous paper (Gagge, 1937), a new physiological variable has been described which gives a measure of the extent of wettedness on the total exposed surface of the body. This variable is defined by the equation

$$(w\mu) = E/A \div [\epsilon(T_S) - rh \times \epsilon(T_A)], \qquad (1)$$

where E is the total evaporation from the body; A is the DuBois area: w represents that fraction of the body surface which, if completely wetted, would produce the observed total evaporative E; μ is the proportionality factor depending on the physical nature of the evaporative processes; $\epsilon(T_S)$ and $\epsilon(T_A)$ are the saturation vapor pressures at skin temperature and air temperature, respectively; (rh) is the relative humidity of the air in terms of a fraction.

The value for $(w\mu)$ above is computed for the body as a whole. By subtracting the evaporative heat loss of the respiratory tract (computed from lung ventilation, see table 1) from the total evaporation, we may obtain a value of $(w\mu)$ for the skin surface alone.

It has been shown (Gagge, 1937) that $(w\mu)$ has a maximum value of 28.5 kilogram-calories per square meter per hour per centimeter of Hg

[3] In our earlier studies, the critical point appeared at 31°C., since, for a *standard time of exposure short of equilibrium*, a slightly higher T_O is required to reach the critical T_S at which positive evaporative regulation begins when a combination of cold air and hot radiation is employed.

pressure for the skin surface. If we then define w as unity or 100 per cent of the maximum possible wetted area, equation (1) becomes

$$w = (E/A) \div 28.5\,[\epsilon(T_S) - rh \times \epsilon(T_A)], \qquad (2)$$

where E now is the net evaporation from the skin surface only. Therefore,

$$28.5\,[\epsilon(T_S) - rh \times \epsilon(T_A)] \text{ or } \xi,$$

is defined as the evaporating power of the ambient air. It is directly proportional to the heat loss by evaporation from a square meter of liquid surface at constant temperature (T_S), when placed in an environment of

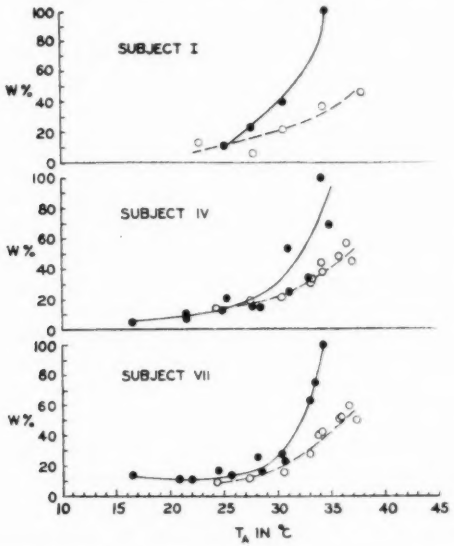

Fig. 2. Percentage of maximum possible area of wetted skin surface (w) in relation to air temperatures. Solid circles, high humidity. Open circles, low humidity.

dry bulb (T_A), and relative humidity (rh). The evaporating power, ξ, and the area of wettedness as a percentage, w, both calculated by the above formula, have been entered in the last two columns of table 1.

In figure 2, this factor of "wetted area," w, is plotted against air temperature for the high and the low atmospheric relative humidities. Here we see a quantitative expression of the factor by which constant evaporation is maintained at a given air temperature with either high or low relative humidity. That there must be a change in sweat secretion to account for a constant evaporation with varying moisture-demands of the atmosphere is, of course, obvious. The closeness of the quantitative results

obtained is, however, striking. At air temperatures under 26.7° for subject I, 29.4° for subject IV, and 30.6° for subject VII, the "wetted area" remains low—about 5 to 20 per cent—irrespective of atmospheric humidity. Above these critical points, however, the values for high and low humidity follow completely different lines. With low humidity, even at air temperatures of 37.8°, the wetted area only reaches 40 to 60 per cent of the maximum possible wetted area. With high humidity at 34.2°C., on the other hand, it rises sharply to 100 per cent.

There is obviously an extremely delicate and perfect mechanism at work which increases the discharge of sweat so as to exactly balance the decreased moisture-demand of a humid atmosphere and thus to maintain evaporative heat loss at a desirable level.

Circulatory changes in the skin in relation to atmospheric humidity. Since secretion of sweat increases with air temperature and with air humidity, it might be expected that the cutaneous blood supply would increase under the same conditions. In a previous study (Winslow, Herrington and Gagge, 1937), we have shown that the conductance of the surface layers of the skin may be computed from the surface area of the body, metabolism, storage, rectal temperature and skin temperature, and represents heat flux through the skin in kilogram-calories for each degree Centigrade fall in temperature per square meter per hour. This factor depends upon the intrinsic character of the tissues of the individual (as modified by various amounts of fat) and upon the blood flow through the skin. The conductance appeared, on analysis of our data, to be materially higher (for a given air temperature) with high atmospheric humidity than with low atmospheric humidity.

General picture of relationship for all subjects. Since the reactions of the three different subjects are, in general, so similar we have combined the results for all three in table 2, computed for all groups of experiments conducted with approximately similar conditions of atmospheric temperature and humidity. Metabolism and heat loss by evaporation have been computed per square meter of body area to make the subjects comparable and values for conductance and wetted area are already on a basis of unit body surface.

The data are divided into six groups, from the standpoint of atmospheric temperature (15.6°–21.0°, 21.1°–26.6°, 26.7°–32.1°, 32.2°–34.9°, 35.0°–37.7°, and 37.8° and over) and into the usual two groups as to atmospheric humidity. As atmospheric temperature rises (both for low and high humidity) we observe the usual rise in body temperature, skin temperature, and evaporation rate. Conductance and wetted area also increase steadily with air temperature, except for conductance at temperatures over 37.8°—a value based on a single group of experiments.

Comparison of the two halves of the table gives us, however, the sig-

nificant approach to our present problem. For air temperatures under 21.0° (group I), we have only high humidity conditions, and for air temperatures over 34.9° (groups V and VI), only low humidity conditions. For the three sets of air temperatures between 21.0° and 34.9°, however, two sets of conditions are available—for low and high relative humidity, respectively, but with metabolism, air temperature, mean body temperature and mean skin temperature for the two humidity groups practically identical (with a single exception as to skin temperature to be noted later).

TABLE 2

Mean comparative data for all subjects classified by atmospheric temperature and humidity

GROUP NUMBER	Number experiments	T_A	RH	M	T_B	T_S	E/A	Cond.	W
	LOW HUMIDITY								
		°C.	per cent	$\frac{kgm.\text{-}cal.}{hr.}$	°C.	°C.	$\frac{kgm.\text{-}cal.}{m.^2/hr.}$	$\frac{kgm.\text{-}cal.}{m.^2/hr./°C.}$	per cent
II	18	24.39	33	47	37.06	32.17	14	15.0	12
III	28	29.11	24	44	37.11	33.28	19	15.0	16
IV	52	33.83	25	46	36.94	35.28	39	27.4	37
V	42	36.44	24	47	37.28	35.39	53	27.7	52
VI	4	37.94	17	40	38.44	35.39	54	11.2	46
	HIGH HUMIDITY								
I	16	18.17	63	47	36.44	30.59	11	21.2	10
II	50	23.78	64	45	36.72	32.33	12	19.4	13
III	56	29.06	60	47	36.94	34.56	20	33.0	26
IV	42	33.89	63	45	36.89	35.39	36	53.2	74

Comparing the figures we note, first, that total evaporation rate is completely unaffected by relative humidity of the atmosphere. The rates are 14 and 12 for group II, 19 and 20 for group III, and 39 and 36 for group IV—the first figure in each case being for low and the second for high atmospheric humidity. In the second place, wetted area is almost identical for both humidities for group II (12–13 per cent) where secretion of sweat is minimal; while for group III the value rises from 16 with low humidity to 26 with high humidity; and for group IV from 37 with low humidity to 74 with high humidity. Conductance of the skin is through-

out higher with high relative humidity (15.0 against 19.4 for group II; 15.0 against 33.0 for group III, and 27.4 against 53.2 for group IV).

Figure 3, plotted from the data in table 2, brings out the close parallelism between the two factors of skin conductance and wetted area. This conductance is striking in view of the fact that these factors are computed from quite independent experimental data. Skin conductance depends on metabolism, storage, body temperature and skin temperature; wetted area on weight loss, skin temperature, air temperature, and atmospheric humidity. When, in addition, one recalls that air temperature and skin temperature were, in general, the same for both high and low atmospheric

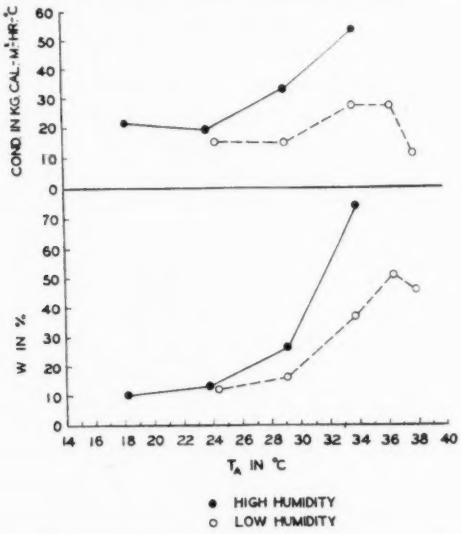

Fig. 3. Mean conductance of body surfaces and percentage of maximum possible area of wetted skin surface in relation to air temperature (for experiments with all subjects studied under corresponding conditions).

humidities, it seems clear that the body responds very definitely to high atmospheric humidity by an increased blood supply to the skin and an increased secretion of sweat—a process nicely adjusted to maintain evaporative heat loss at the desirable level.

Two alternatives suggest themselves as possible explanations of the mechanism of this process. It may be that slight rises of skin temperature do actually occur which stimulate sweat secretion and lead to a prompt return of skin temperature to normal. Or the moisture content of the skin as influenced by evaporation may have a stimulating influence.

Whether the fall in conductance and sweat secretion for group VI is

significant we are not certain. As stated above, there was but one group of experiments under this condition, with an abnormally low metabolism.

Limits to the cooling power of the evaporative mechanism. The power of the body to adjust its heat losses in a hot environment has, as we have seen before, its definite upper limits and the data already obtained make it possible to calculate these limits with a reasonable degree of precision.

The heat interchanges of the body for a condition of equilibrium may be expressed as follows:

$$M = E + (R + C), \tag{3}$$

when M = Metabolism,

and E = Total evaporative heat loss,

$(R + C)$ = Combined heat loss by radiation and convection.

From our previous studies (Winslow, Herrington and Gagge, 1937) we know that

$$(R + C) = K_o(T_S - T_o), \tag{4}$$

where K_o = Environmental constant,

and T_S = Skin temperature,

T_o = Operative temperature.

With wall and air of equal temperature and standard air movement (17 feet per minute), T_o is the same as T_A (air temperature).

Furthermore, if we divide all factors by the DuBois area we can express our results per square meter of body surface.

Equation (3) then becomes, per unit area,

$$M' = E' + K_o'(T_S - T_A). \tag{5}$$

We know from our previous work that M (metabolism per square meter of body surface) is 47 kilogram-calories per hour[4] and that K_o is 8.45. With these data, and transposing, we have

$$E' = 47.0 - 8.45\,(T_S - T_A). \tag{6}$$

From equation (1) we have

$$E' = (w\mu)[\epsilon(T_S) - rh \times \epsilon(T_A)]. \tag{7}$$

Combining (6) and (7) and solving for the humidity (rh) we have

$$RH = 100/\epsilon(T_A)\left[\epsilon(T_S) - \frac{47.0 - 8.45\,(T_S - T_A)}{(w\mu)}\right]. \tag{8}$$

Equation (8) describes the interrelationship between the four variables, T_S, T_A, RH (as a percentage) and $(w\mu)$, under all conditions where regulation may be maintained, i.e., when storage is zero.

[4] Mean metabolism for young male subjects in semi-reclining position as in our experiments.

In a previous communication, one of us (Gagge, 1937) has shown that at the upper limit of regulation by sensible perspiration, ($w\mu$) has a maximum and constant value of 29.9 kilogram-calories per square meter per hour per centimeter of Hg vapor pressure for the total body. If we assume that at the upper limit the skin temperature is 35.6°C., the mean temperature corresponding to a 100-per cent wetted area in table 1 for our three subjects, we may now substitute these values in (8) and have

$$RH = 100/\epsilon(T_A)\left[4.33 - \frac{47.0 - 8.45\,(35.6) - T_A)}{29.9}\right],$$

which equation may simplify to

$$RH = 100/\epsilon(T_A)\left[\frac{45.3 - T_A}{3.54}\right]. \qquad (9)$$

From equation (9) one may compute for any air temperature the relative humidity which will correspond to the upper limit of evaporational cooling. This has been done in table 3.

TABLE 3

Upper limits of evaporative regulation at 17 ft/min. air movement

DRY BULB TEMPERATURE	RELATIVE HUMIDITY	WET BULB TEMPERATURE
°C.	*per cent*	°C.
45.3	0	16.6
42.5	13	21.1
40.0	28	24.4
37.5	47	27.8
35.0	70	30.0
32.5	100	32.5

These figures correspond reasonably well with the findings of McConnell, Houghten and Yagloglou (1924) that saturated air at 32.2° is the "upper limit of man's ability to compensate for atmospheric conditions" in still air. They are also in close accord with the reports of Cadman and Haldane (British Departmental Committee, 1909). Cadman states that at 29.4° wet bulb temperature, the body temperature invariably rises, while at 33.9° wet bulb, "one is in a terrible state"; Haldane, that at 31° to 32° wet bulb "in fairly still air the body temperature begins to rise, even in the case of persons stripped to the waist and doing no work; and when air is saturated this rise continues until symptoms of heat stroke arise."

SUMMARY

In the present paper we have:

1. Applied a new procedure for estimation of the total effective sweat secretion from the human body surface.

2. Demonstrated that the discharge of sweat exhibits a close adaptation to the evaportive moisture demands of the environments (as those demands are conditioned by both the temperature and the relative humidity of the atmosphere) so that evaporative heat loss from the body maintains the level necessary to dissipate the heat produced by metabolism.

3. Shown that the increased excretion of sweat associated with high atmospheric humidity at any given air temperature, is accompanied or preceded by an increased cutaneous blood supply—for which process the initial stimulus must presumably be peripheral rather than central, since body temperature does not change appreciably in the process while even skin temperature alters but slightly and temporarily.

4. Indicated on theoretical grounds the upper limits of temperature and relative humidity beyond which this regulative process fails.

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THE EFFECTS OF HYSTERECTOMY ON MAMMARY GLAND DEVELOPMENT IN A RABBIT

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Many factors leading to mammary-gland development and lactation have been demonstrated but these do not necessarily lead to the production of secretory activity.

Allen (1) states that estrin produces marked elongation in the ducts and an increase in number of primary buds of the alveoli in rabbits. Loeb (2) showed that estrin injected into cryptorchid male guinea pigs produced mammary-gland development. Nelson and Smelser (3), while working with the same animal, were able to produce lactating breasts with estrin and anterior pituitary extract. They, therefore, assumed that the lutein hormone is not an essential factor. In opposition to this, Turner and Frank (4) are of the opinion that estrin produces duct development and not alveolar development. However, if corpus luteum was added there followed the development of alveoli and milk secretion.

Selye, Collip and Thomson (5) have shown that in the rat hypophysectomy during lactation leads promptly to cessation of milk secretion and that Cesarean section shortly before term regularly induces lactation, but fails to do so if the pituitary gland has been removed. They concluded that the sudden decrease in the amount of estrin in the circulation is a stimulus for the production of this hormone, prolactin. The same authors (6) suggest that the pregnant uterus, like the hypophysis, supplies a stimulus to the mammary glands. In 1932, Loeb (2) found a marked prolongation of the life and function of the corpus luteum in pseudopregnant hysterectomized guinea pigs. Normally, degeneration of the corpus luteum takes place in 14 days; however, he demonstrated a functional corpus luteum 60 days old. The corpus luteum of pregnancy lasts 65 days. He found mammary gland development quite marked with mytotic proliferation as late as 74 days after the last ovulation of a hysterectomized guinea pig. Asdell and Hammond (7) found a somewhat similar result in rabbits. Normally, a functional corpus luteum in a pseudopregnant rabbit lasts 16 days, but they found its functional activity present 24 to 29 days after initial copulation. The corpus luteum of pregnancy in rabbits lasts 30 days. Although they found the corpora

lutea functional as inhibitors of follicular ripening, yet no more growth takes place after hysterectomy than is normal during pseudopregnancy. They conclude that it is an extra-ovarian stimulus which carries the mammary development beyond that of pseudopregnancy.

Sessums and Murphy (8) found that hysterectomized rabbits failed to have an estrous cycle after the period of pseudopregnancy. They also state that there is a frequent cystic degeneration of ovaries following hysterectomy.

We have studied mammary gland development in hysterectomized and non-hysterectomized rabbits. Our method of approach has been total mounts of the mammary gland biopsy.

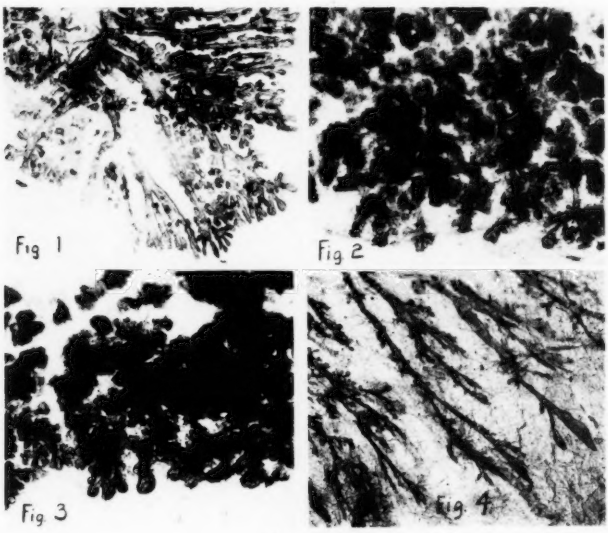
The procedure was as follows: virgin, immature rabbits were isolated. A hysterectomy was performed on one-half of the rabbits while the other half were used as controls. When in heat, a condition of pseudopregnancy was produced by allowing copulation with a vasectomized buck, by intravenous injections of prolan or by copulation with a normal buck and the use of contraceptive jelly in the vagina. Either on the same day or the following day, a laparotomy was done to prove the rupture of the ovarian follicle and the formation of corpora lutea. A biopsy of the breast was taken at the same time. Biopsies were then taken every three to four days thereafter until the end of the pseudopregnancy. Some hysterectomized and non-hysterectomized rabbits were carried through as many as three periods of pseudopregnancy. The end of the pseudopregnancy was determined by all of the following criteria: 1. Definite desire for the buck. 2. Characteristic bluish-purple color of the vagina. 3. Actual mating with definite rupture of ovarian follicles.

Experimental. Eight hysterectomized and a similar number of non-hysterectomized virgin rabbits were followed. It was expected that the glands would show a marked atrophy soon after the termination of the pseudopregnancy. However, it has been found that there is no marked atrophy of the glands which can be utilized as an end point for the experiment. Thus, the duration of pseudopregnancy in this work is based on the return of estrus and not on the study of the mammary gland. We found pseudopregnancy to last 18 days in non-hysterectomized and 25 days in hysterectomized animals.

By observing the gland within 24 hours after ovulation we can state that only duct formation was found to develop before follicular rupture and development of corpus luteum. Figure 1 is the mammary gland of a normal rabbit showing primary duct proliferation within twenty-four hours after first ovulation. We feel this is an estrin reaction only. As early as the third day after rupture of the follicle there was a marked lobule formation in both non-hysterectomized and hysterectomized rabbit mammary glands probably from progestin stimulation. Figure 2 is the

mammary gland of a normal rabbit 3 days after the first ovulation, showing lobules (?corpus luteum reaction). Figure 3 is the mammary gland of a hysterectomized rabbit 3 days after first ovulation, showing no change in gland architecture from that of a normal rabbit.

In a control series of experiments we found that prolonged treatment with estrin in an oöphorectomized virgin rabbit produced duct development only. Figure 4 is a mammary gland of a bilateral oöphorectomized rabbit following weekly injections of estrin for a period of 3 months. Duct formation and end-bulbs but no alveoli are present. This confirms the work of other investigators.



Figs. 1–4

Grossly the hyperemia associated with glandular stimulation disappears at the end of the pseudopregnancy in both groups. There is also an apparent gross atrophy of both groups at this time. However, the stained preparations do not demonstrate this atrophy. End-bulbs are present in most glands at any time during pseudopregnancy, indicating active proliferation. The degree of gland development in the normal and in the hysterectomized animal is strikingly similar in spite of difference in length of pseudopregnancy. Even though hysterectomy nearly doubles the span of corpus luteum there is no evidence in our biopsies that this leads to an increased degree of development of the mammary gland.

We have been able to carry four hysterectomized rabbits through

three periods of pseudopregnancy, observing definitely that there was follicle rupture in each case. An increase in the size of the gland was noted in each successive pseudopregnancy, but no difference in gland architecture. The glands were apparently maintained at a certain level without the production of milk. At autopsy no evidence of ovarian cystic degeneration was observed.

CONCLUSIONS

1. Hysterectomy delays degeneration of corpus luteum and causes a prolongation of pseudopregnancy.

2. Estrin regulates mammary-duct development while corpus luteum probably affects alveolar development.

3. Prolongation of pseudopregnancy causes a hyperemic condition of the mammary gland but has no apparent effect upon the gland architecture itself.

4. Hysterectomy does not prevent further estrous cycles in rabbits.

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ELECTRIC RESPONSES OF THE BRAIN STEM TO UNILATERAL AUDITORY STIMULATION

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Recent research in the electrophysiology of audition has been primarily concerned with the cochlear response (Davis, Derbyshire, Lurie and Saul, 1934; Wever and Bray, 1936a, 1936b; Davis, 1935; Culler, 1935; Hallpike and Rawdon-Smith, 1934a, 1934b; Fowler and Forbes, 1935; Stevens, Davis and Lurie, 1935; Stevens and Davis, 1936). Derbyshire and Davis (1935) have examined the responses of the eighth nerve to tones and have furnished a useful analysis of the complex responses which result from stimulation with clicks. We have attempted to extend this kind of investigation to include the description of the responses of the auditory tracts of the brain stem. There have been a number of preliminary investigations of the activity of these tracts (Saul and Davis, 1932) but those earlier studies were essentially exploratory.

The anatomical description of the cochlear nuclei given by Lorente de Nó (1933) would lead us to expect modification of the eighth nerve pattern by the synaptic regions of the cochlear nuclei and superior olives. Lorente de Nó says:

> The cochlear nuclei appear now to be even more complicated than the retina or olfactory bulb, which are, according to Cajal's description, the primary nuclei of highest differentiation in the nervous system. Each cochlear fiber has connections with very numerous cells grouped in no less than thirteen regions of specific structure in which different types of neurons are found. . . . Although numerical data are not yet available, it is already safe to state that in the primary cochlear nuclei no less than forty or fifty types of neurons are present, and that each cochlear fiber establishes connections with many hundreds, perhaps thousands of cells (1933, p. 22).

Our investigation is concerned with the kinds of modification which result from passage through these centers.

APPARATUS. Our apparatus was essentially the same as that used by

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Derbyshire and Davis (1935)[4] in their study of the action potentials of the auditory nerve. A dynatron oscillator produced sine waves of frequencies from 20 to 12,000 cycles per second. The output of this oscillator was sent directly to loud-speakers when tonal stimulation was desired. Clicks at a frequency of 60 per second were obtained by sending the output of a thyratron oscillator into the loud-speakers.

METHOD. Cats were used under moderate avertin (tribromethanol)[5] anesthesia. (The usual dose of avertin was 240 mgm. per kilo of body weight, injected intraperitoneally. We found it advisable to administer the total dose in two or three separate injections over a period of about 30 minutes, in order to avoid respiratory or cardiac failure.) The bulla and round window were exposed bilaterally and one cochlea was destroyed to insure monaural stimulation at all intensities. An opening was made in the skull over the occipital and parietal lobes. It extended almost to the longitudinal sinus and left intact the insertion of the dura. The opening was enlarged caudally until the cerebellar cavity was reached. The skull was then removed from above the lateral lobe of the cerebellum back to the insertion of the dura and a triangular portion of the tentorium on one side was removed. The dura was incised and removed to expose the occipital lobe and the lateral lobe of the cerebellum. The cerebral hemisphere was then retracted forward and the inferior colliculus and brachium were exposed. When hemorrhage was not too abundant, it was possible to cut the remaining meningeal covering and to expose the medial geniculate body and superior colliculus anteriorly and the inferior colliculus and lateral lemniscus posteriorly. This allowed us to place concentric electrodes in the medial geniculate body, brachium, inferior colliculus and lateral lemniscus. Concentric electrodes were also inserted through the cerebellum into the trapezoid fibers and their position was determined later by microscopic examination.[6]

Homolateral and contralateral exposures were made on alternate experimental days. In all experiments a wick electrode applied to the round window permitted us to obtain a record of the end-organ response, which served as a zero point for latency measurements.

RESULTS. 1. *Stimulation by tones.* Synchronized responses to tones of frequencies up to about 2500 cycles per second can be obtained from fibers

[4] The original construction and subsequent development of this apparatus was assisted by grants from the DeLamar Mobile Research Fund of the Harvard Medical School, from the Josiah Macy, Jr. Foundation, from the American Otological Society, and from several anonymous donors.

[5] The avertin (tribromethanol in amylene hydrate) was generously furnished by the Winthrop Chemical Company.

[6] Dr. M. Votquenne, C. R. B. Fellow in Experimental Surgery, has kindly assisted us in making the microscopic examinations.

of the trapezoid body. When frequencies higher than 2500 per second are employed the responses are asynchronous. Responses to low frequencies are often obtained with stimulating intensities 10 to 20 db lower than the threshold for round window recording.

We have obtained more extensive data from positions in the auditory tracts at the level of the inferior colliculus. From this level synchronized responses are obtained only with stimulating frequencies below 1500 cycles per second. The upper limit of synchronous response is frequently as low as 1000 cycles per second. Figure 1 compares an audiogram taken from this position with an audiogram taken from the round window in the

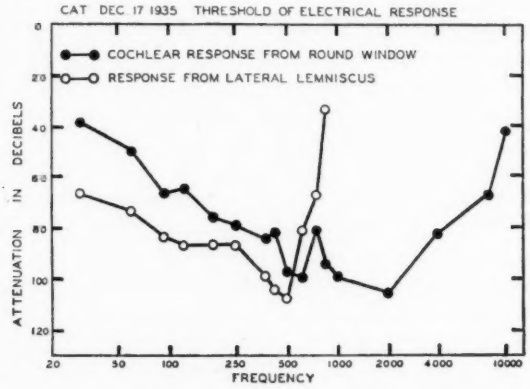

Fig. 1. Comparison of thresholds of electric response from round window (end-organ response) and from lateral lemniscus (third-order neurones). Intensity of sound is expressed in terms of decibels of attenuation below one volt; the greater the amount of attenuation, the weaker the sound. Note that responses to frequencies below 500 are obtained from fibers of the lateral lemniscus at a level of intensity 10 to 25 db lower than is required to elicit a measurable end-organ response at the round window. The break at 500 is typical of many experiments; above this frequency asynchronization occurs unless high intensities are used. Above 1000 asynchronization is complete.

same preparation. We see that the audiogram from the lateral lemniscus shows a considerably lower threshold for low frequencies than the round window audiogram. From the results of Hallpike and Smith (1934a) and of Stevens, Davis and Lurie (1935) we know that recording from the apex of the cochlea yields lower thresholds for low frequencies than does the round window. Since the lowest thresholds which we have obtained from the tracts of the medulla and midbrain are about as low as those which we should expect to obtain from a combination of round window and apical recording, we are inclined to believe that the true thresholds for eighth nerve and auditory tract responses are probably reached at levels of stimulating intensities which give a cochlear response of one or two microvolts.

As the intensity of the stimulating tone is increased, the amplitude of the response increases. Figure 2 shows how the amplitude of the recorded response increases with increasing intensity of stimulation. The variety of curves shown is typical of those experiments in which complete studies of this kind were carried out. The irregularities probably depend fundamentally on the relatively localized pick-up by the concentric electrodes from a random position in a complicated nervous tract.

2. *Stimulation by clicks.* a. *Responses from the trapezoid fibers.* In most cases we have stimulated with clicks at a frequency of 60 per second. With suitable electrode placements in the medulla we obtain a response to

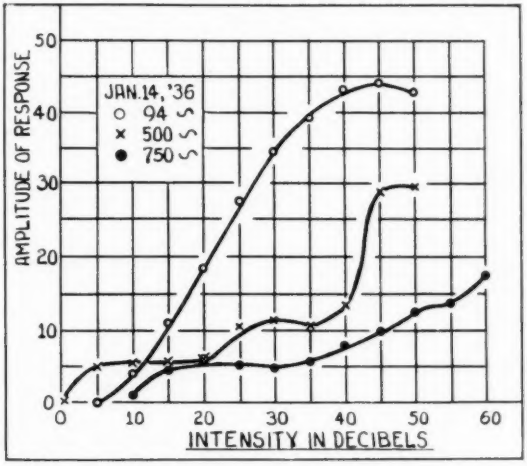

Fig. 2. Amplitude of electrical response recorded from fibers of lateral lemniscus as a function of intensity of stimulation. In this figure the intensity is expressed in terms of decibels above an arbitrary "threshold" level. The increase in amplitude shows that additional fibers are being activated with increasing intensity of stimulation. The irregularities in some of the curves are probably due to the fairly localized "pick-up" by concentric electrodes from a single random position in a complicated tract.

each click. The response is usually complex, but it is often possible to analyze it into several components by stimulating the ear simultaneously with tones of certain frequencies. Figure 3 shows how separate components of the click response may be "masked" by certain bands of frequencies. We owe this method of analysis to Derbyshire and Davis (1935) who used it in the analysis of responses recorded at the round window to these same stimuli. According to their interpretation, this masking phenomenon is due to competition for the same nerve fibers by two stimuli which arrive almost simultaneously. They have shown that the degree of masking depends upon the phase relation of click to tone, and that

separate waves of the click response are masked selectively by simultaneous stimulation with masking tones of certain frequencies. We find that in the medulla, as in the eighth nerve, responses to clicks have early components masked by higher frequencies and later components masked by lower frequencies.

The earliest waves recorded from the trapezoid body appear about 2 to 3 msec. after the beginning of the cochlear response, and about 1.3 to 2.0 msec. later than the correspondingly-masked action potential waves in the eighth nerve. This latency decreases with increase of intensity of stimulation until a minimum is reached at an intensity 40 to 45 db above threshold. Over this intensity range the shift in absolute latency may

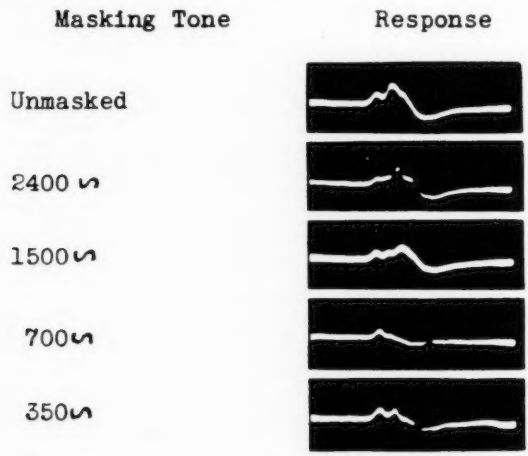

Fig. 3. Oscillograms of the responses recorded from fibers of the lateral lemniscus to clicks, showing masking. Note that different frequencies of masking tone affect different components of the click response.

be as great as 0.7 msec. This decrease in latency seems to occur even with waves which have widely different thresholds. Derbyshire and Davis found that the latency of the eighth nerve responses to clicks decreased by about 0.3 msec. over this intensity range, which leaves about 0.4 msec. of the total latency shift to be attributed to the synapses in the cochlear nucleus. (See figs. 4 and 5.)

In cases where the first component of the complex wave is well separated from the later ones we have made measurements of amplitude as a function of intensity. With a single electrode placement, the recorded response reaches a maximum at an intensity level 30 to 40 db above the "threshold" for that position. We believe that this maximum is due to

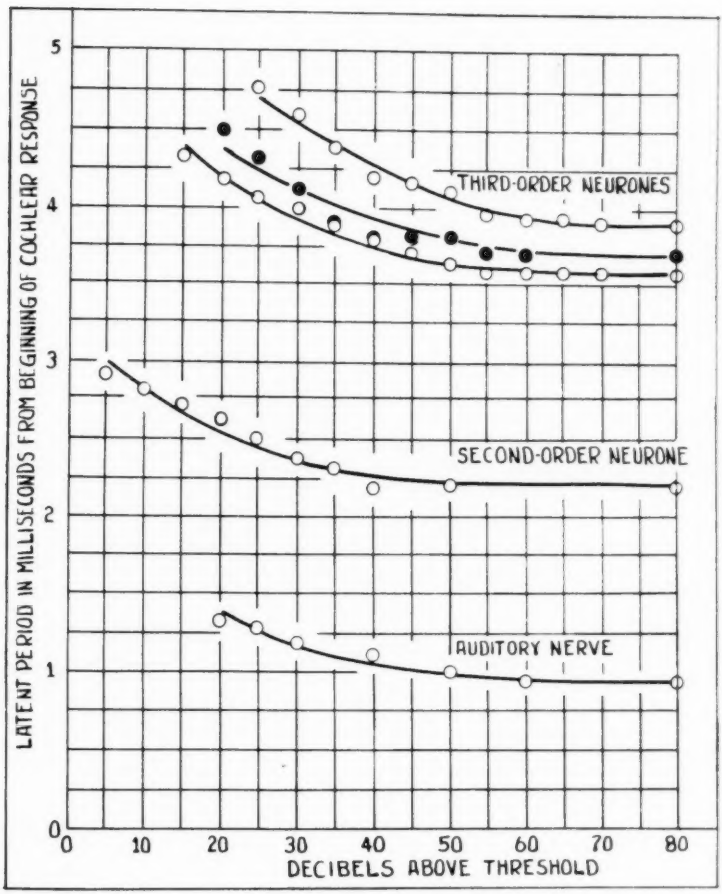

Fig. 4. A comparison of latency-intensity measurements from five recording positions in a single preparation. The auditory nerve responses were recorded from the round window (see text and also Derbyshire and Davis, 1935). The second-order neurones were fibers of the trapezoid body and the third-order neurones were fibers of the lateral lemniscus. Note that the latent period decreases with increasing intensity until it reaches a final minimal value and that the amount of latency decrease is greater in the second- and third-order neurones than in the fibers of the auditory nerve. The reduction in latency in the fibers of the auditory nerve is probably due to stimulation at an earlier phase of the sound wave (see Derbyshire and Davis, 1935). The "threshold" in this figure is arbitrarily chosen as an intensity level slightly lower than that which elicited responses from the trapezoid fibers.

the method of recording and that additional fibers are activated at higher intensity levels, for another electrode placement in the same preparation may show a considerably higher "threshold" and reach its maximum of response only at a correspondingly higher intensity of stimulation (fig. 6).

b. *Responses from the midbrain.* We inserted concentric electrodes at several points on the surface and into the depths of the inferior colliculus. We obtained most satisfactory responses from deep posterior placements, where we were probably recording from the lateral lemniscus in the region where a compact bundle of fibers enters the inferior colliculus. Responses to clicks were obtained from these fibers at intensities 10 to 20 db lower than the usual round-window "thresholds." These responses increased regularly in amplitude with increasing intensity until maxima were

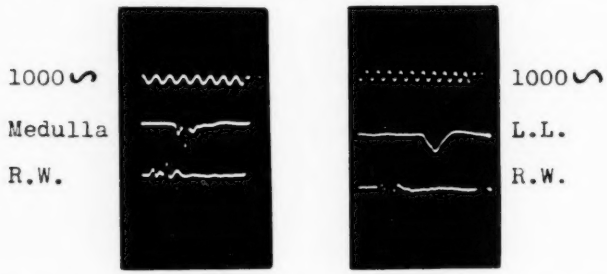

Fig. 5. Oscillograms illustrating the method employed in latency measurement. The speed of sweep is indicated by the time line (1000). The electrical signal into the loud-speaker starts the sweep. The stimuli were in each case clicks at 60 per second. After a short delay required for conduction of the sound the basilar membrane is activated and this is accompanied by the cochlear response (recorded in this case from the R.W.). If we record from the trapezoid fibers or from the lateral lemniscus, the additional delay may be easily measured.

reached, as in the trapezoid fibers. The earliest waves recorded at the level of the inferior colliculus appeared 2.3 to 3.1 msec. later than the corresponding waves from the fibers of the eighth nerve. This latency decreased with increasing intensity of stimulation, as in the case of the trapezoid fibers. The total shift in absolute latency was sometimes as great as 1.0 msec., as compared with 0.7 msec. in the trapezoid fibers and 0.3 msec. in the fibers of the eighth nerve.

The waves recorded from the midbrain usually consist of a number of components which can be masked selectively. When we record from the anterior pole of the inferior colliculus, they are masked by rather high-frequency stimulation (above 2000 cycles per sec.). At the center of the inferior colliculus the responses are larger, but the masking frequencies are of about the same range. With deep posterior placements the responses are large and quite complex. The earlier part is still masked with high frequencies, but there are later components which are masked by

frequencies below 1000 per second. We have not observed components masked by such low frequencies in waves recorded from the anterior and medial portions of the inferior colliculus. It is possible to interpret these results as due to a crude projection of the basilar membrane on the inferior colliculus. Fibers responding to all frequencies may pass into the posterior portion of the inferior colliculus, but, while fibers responding to high frequencies may pass forward to terminate in the medial and anterior portion of the colliculus, those fibers which respond to low frequencies may terminate in the posterior portion.

In a few experiments we made consecutive electrode placements, going from the inferior colliculus along the lateral lemniscus toward the cochlear nucleus. Just posterior to the inferior colliculus the responses were the

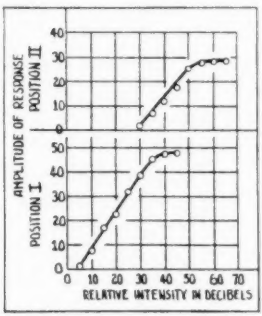

Fig. 6. Amplitude of response, recorded from fibers of the lateral lemniscus (third-order neurones), as a function of increasing intensity of a click. The apparent maximum obtained at position I appears to be due to localized recording and is no indication that additional fibers are not activated at higher intensities, for from another position in the same preparation we obtain a response which has a considerably higher threshold and which continues to grow in amplitude over a correspondingly higher range of intensity.

same as those recorded from the posterior pole of this center. With placements about 1.5 mm. posterior, however, we obtained the typical midbrain response and small preceding waves. With a placement still more posterior only the small preceding waves appeared. With a very posterior position (in the edge of the cochlear nuclei) we obtained quite large responses which were similar in latency and amplitude to those usually observed at the level of the trapezoid fibers. It seems possible that at the first placement we were recording from third-order neurones beyond the nucleus of the lateral lemniscus; at the second position we were in its immediate vicinity, so that we could record from both third-order and second-order neurones; in the third position we were in the posterior edge of this nucleus, recording from a few second-order neurones; and in the fourth position we were near the acoustic tubercle, and recording optimally from only second-order neurones.

c. *Responses from brachium and medial geniculate body.* With electrode placements in the brachium and medial geniculate body, we succeeded occasionally in obtaining responses of small amplitude. In most cases no responses were obtained, although the electrode position was verified by post-mortem examination. We attribute our frequent failures to obtain responses and the small amplitude of those occasionally obtained to a partial or complete block by the anesthetic (tribromethanol) of the auditory pathways below this level. In those cases in which we obtained responses, the first wave (masked by high frequencies) usually appeared about 4 to 5 msec. later than the corresponding wave in the eighth nerve. With increasing intensity of stimulation the amplitude changed very little but the latency decreased by about 1 msec. Thresholds for these small responses were sometimes almost as low as the round-window threshold.

d. *Effect of supplementary anesthetics.* In these experiments the cats were under moderate or deep "avertin" anesthesia. We observed the action of ether and chloroform as supplementary anesthetics on the large responses obtained from the lateral lemniscus and trapezoid fibers. The end of the tracheal cannula was inserted into a test-tube, in the bottom of which was a wad of cotton saturated in ether or chloroform. The amplitude of the electric response was measured at half-minute or minute intervals, or at shorter intervals during very rapid changes. Supplementary ether produces a progressive reduction in the amplitude of responses recorded from trapezoid fibers until the time when the respiratory reflex is lost. Asphyxia then produces a very rapid shrinkage of the response and it disappears completely in less than two minutes. The effect of chloroform is similar to but more rapid than that of ether. The decrease in response is about 1 to 4 per cent per minute until it has decreased by about 50 per cent of the original amplitude. The sudden drop due to asphyxia usually occurs at this time. At the level of the inferior colliculus the initial phase of diminution in amplitude is more rapid than at the trapezoid level; the drop for ether is 3 to 11 per cent per minute and for chloroform from 16 to 66 per cent per minute. At this level we usually observed that the response almost, or entirely, disappeared before spontaneous respiration ceased. It seems then that the olivary complex is affected by these anesthetics at about the same stage as the respiratory center. The cochlear nucleus, on the other hand, seems more resistant to anesthesia than is the respiratory center.

When supplementary anesthesia is stopped recovery occurs in two phases. In the initial phase the response from the trapezoid body reaches in less than two minutes the level at which it had been when spontaneous respiration ceased. In the second phase the amplitude increases more gradually until it reaches its original value. The first visible response is often simultaneous with the first spontaneous respiratory movement. At the level of the inferior colliculus the two phases are not as distinct as

at the trapezoid level, but it is usually possible to observe a faster initial recovery followed by a slower increase to the original level. (For further details see Coppée and Kemp, 1936.)

Discussion. 1. *The location and function of synapses.* The auditory tracts appear to be interrupted by synapses in the cochlear nuclei and in the superior olivary complex. When we record from electrode placements in the trapezoid fibers we find that the latencies (measured from correspondingly masked eighth nerve potentials) usually fall into our shorter range of 1.0 to 1.5 msec. Occasionally we obtain double waves which are masked simultaneously. The second wave shows a latency of our longer range. From the midbrain, however, we invariably find latencies of about 2.0 to 2.8 msec. (measured from correspondingly-masked action potentials in eighth nerve fibers). Because no intermediate latencies have been observed, we infer that all fibers which reach the level of the midbrain have been interrupted at least twice—i.e., once in the cochlear nuclei and again in the olivary complex, to which we think the nucleus of the lateral lemniscus belongs.

Some anatomists have stated that the central acoustic tract runs without interruption from the cochlear nuclei to the medial geniculate body. Since we have never found extremely short latencies at the midbrain level, we must either 1, doubt the existence of such a tract; 2, postulate a double synaptic delay for its fibers in the cochlear nuclei, or 3, entertain the possibility that it fibers have been inactivated by the anesthetic. Some anatomists do not mention such a tract.

When reasonable values for conduction time have been subtracted, the minimal values for synaptic delays appear to be about 0.8 msec. for the cochlear nucleus and about 0.8 msec. for the olivary complex. (See fig. 4.) These are of the same order as those measured by Lorente de Nó (1935) in the oculo-motor nuclei.

Since the decrease in latency with increasing intensity of stimulation is usually greater in the second and third order neurones than in the first order neurones, we think that some degree of spatial summation may occur in the cochlear nuclei (cf. Eccles and Sherrington, 1930).

The rather critical upper frequency limits for synchronization which are encountered when recording from these second- and third-order neurones are undoubtedly determined by the properties of the synapses. Above the limiting frequencies, asynchronization due to temporal dispersion of the impulses in separate fibers becomes so great that the frequency of the stimulation can no longer be detected. The upper frequency limit seems to correspond to the upper limit at which the neurones can conduct impulses without alternation. Apparently temporal dispersion is increased by differences in synaptic delays when there is no longer a 1-to-1 response to impulses succeeding one another at regular intervals.

2. *Auditory theory.* Our results on the selective masking of separate

components of the complex responses to clicks may be explained most easily in terms of an extension of the place theory. This theory holds that a sound wave of a particular frequency sets a selected area of the basilar membrane into vibration, the result being that impulses travel up the auditory nerve fibers which correspond to that area. It appears that certain second and third order fibers may be occluded (masked) by activation of certain portions of the basilar membrane and not by the activation of other regions. (See fig. 3.) This means that those second and third order fibers ordinarily carry impulses from certain portions of the basilar membrane and not from others. Advocates of the place theory usually suggest that the width of the vibrating basilar segment increases with increasing intensity of stimulation, thus bringing about the activation of additional fibers of the auditory tracts. Our observations on increasing amplitude of electric responses with increasing intensity of stimulation support this theory of the physiological correlate of loudness.

3. *Thresholds.* The threshold of nerve response is obviously more significant physiologically than any thresholds of the cochlear (sense-organ) response.

It appears that, if the electrodes are favorably placed, neural thresholds in the cat are reached with stimulating intensities which are at about the level of the human audible threshold. Cochlear responses to these intensities may be detected if the grid electrode is in contact with the apex when low tones are employed and in contact with the round window when high tones are employed as stimuli. Still weaker cochlear responses may yet be detected, but they can be of no significance for hearing if they fail to initiate nerve impulses.

4. *Bilateral representation of each cochlea.* Representation of each cochlea seems to be bilateral in the lateral lemniscus. Thresholds, latencies, and maximal amplitudes attainable are not significantly different when homolateral and contralateral placements are compared.

SUMMARY

Action potentials from the trapezoid fibers and the lateral lemniscus of the cat have been recorded by means of a resistance-capacity coupled amplifier and a cathode-ray oscillograph. With tones as stimuli, synchronized responses are obtained from the trapezoid fibers at frequencies up to 2500 per second. The increase in amplitude of responses with increasing intensity of stimulation is quite complex, but indicates that activation of additional fibers is probably the physiological basis for increasing loudness. In the lateral lemniscus synchronized responses are obtained at threshold intensities with frequencies below 500 per second; if higher intensities are used synchronized responses may be observed with frequencies up to 1000 per second. Above this limit the responses are

asynchronous. The neural thresholds appear to be reached at intensities which yield just measurable cochlear responses (1–2 μ v) when the active cochlear electrode is on the apex for low frequencies and on the round window for high frequencies.

A more extensive investigation has been made with click stimuli. Responses from the trapezoid fibers appear 1.3 to 2.0 msec. after corresponding action potentials in the eighth nerve fibers, while responses from the fibers of the lateral lemniscus appear about 2.3 to 3.1 msec. after eighth nerve potentials. When suitable values for conduction time are subtracted, synaptic delays of 0.8 msec. in the cochlear nuclei (first synapse) and 0.8 msec. in the superior olivary complex (second synapse) are assigned. Greater decrease in latency on increasing stimulation in the second and third order neurones than in the eighth nerve fibers has been interpreted as possibly due to spatial summation in these two synaptic regions.

Separate components of the complex responses to clicks may be masked (occluded) by simultaneous stimulation with tones of the correct frequencies. The best explanation of this fact seems to be in terms of an extension of the place theory.

Ether and chloroform as anesthetics supplementary to avertin bring about reductions in amplitude of responses. The olivary complex seems to be about as susceptible to these anesthetics as is the respiratory center, while the cochlear nuclei are less susceptible.

We wish to express our appreciation to Prof. Hallowell Davis for many valuable suggestions and for the facilities which he has placed at our disposal.

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ELECTRIC RESPONSES OF THE BRAIN STEM TO BILATERAL AUDITORY STIMULATION

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The first paper of this series (Kemp, Coppée and Robinson, 1937) was an account of the electrical responses from various positions in the auditory tracts of the cat's brain stem, following monaural auditory stimulation. In the present investigation we have compared homolateral with contralateral responses and have observed the effects of binaural stimulation. Threshold measurements, comparisons of latency and amplitude, and observations on synchronization and masking provide evidence pertaining to the problems of binaural interaction and auditory localization.

While there has been a good deal of theorizing about the underlying physiology of binaural phenomena, electrical recording of responses to binaural stimulation has been confined to a few essentially exploratory observations by Saul and Davis (1932).

METHODS. Our general operative and recording technique was similar to that previously described (Kemp, Coppée and Robinson, 1937). In the present experiments, however, both bullae were opened and electrodes placed on both round windows. The inferior colliculus and lateral lemniscus were exposed on one side, or in some instances on both sides, and concentric electrodes were placed in the auditory tracts at the midbrain level. In other experiments, concentric electrodes were inserted through the cerebellum into the medulla as in the previous investigations.

In order to insure equivalent stimulation of the two ears, we used two carefully matched speakers (Western Electric 597-B). These were placed in a box insulated for sound, and their outputs were delivered to the ears of the cat through two similar hose and side-tube connections. Discharges from a dynatron or a thyratron oscillator were sent through two parallel attenuating networks into these loudspeakers, allowing independent variation of the intensity of the stimuli to the two ears. By varying the length of a slide tube inserted into the hose connection to one of the ears we could stimulate one ear before, simultaneously with, or after the other. We utilized the cochlear response from each ear as a control on the wave

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form and time relations of the stimuli. Thus we could correct for over-all differences in thresholds at the two ears (due to differences in middle-ear conduction, inner ear condition, or to possible differences in our stimulating apparatus). Comparison of the two round window thresholds assured us that the stimuli were not only physically but physiologically equivalent. Preparations in which the sensitivity of the two ears was markedly different, or in which abnormalities of both ears were found, were rejected.

RESULTS. 1. *Alternate stimulation of the two ears.* During our previous experiments we observed that responses from homolateral and contralateral tracts were generally similar in threshold, latency, and amplitude. Those observations, however, were from homolateral and contralateral operations, carried out on different days and on different cats. In the present experiments we recorded from a position in the midbrain or medulla, stimulating homolaterally and contralaterally in alternation. We first determined the approximate threshold for cross-stimulation of the supposedly "unstimulated" ear. (The cross-stimulation was probably due to air conduction rather than bone conduction since the bullae were necessarily open.) This threshold was determined in each experiment in order to make certain that binaural effects were really being compared with *monaural* effects. Only with intensities less than 30 to 40 db above threshold could we be resonably certain of monaural stimulation.

Latencies were measured from right and left ears to particular positions in the lateral lemniscus. When the intensity level above threshold was maintained equal for both sides, the latency differences observed were small and random in direction. Apparently, homolateral and contralateral responses pass up the lateral lemniscus on both sides in exactly the same time relation as that in which stimulation of the two ears has occurred. In other words, time relations are maintained precisely in each lateral lemniscus for both homolateral and contralateral components (see fig. 1A).

The amplitude of response varies with intensity of stimulation in a similar manner for both homolateral and contralateral recording. For any particular position of the electrodes, either the crossed or the uncrossed response may increase more rapidly or reach a higher amplitude, but these variations are apparently random, and probably depend upon the proportion of crossed and uncrossed fibres from which we happen to be recording.

When tonal stimulation is used a placement which gives synchronous responses for homolateral stimulation also gives synchronous responses for contralateral stimulation. Upper frequency limits for synchronization are very nearly equal for both sides.

2. *Simultaneous stimulation of both ears.* In the case of binaural stimulation, the latency is equal to that observed for either monaural contra-

lateral or homolateral stimulation. No appreciable decrease in latency is brought about by stimulation of the two ears at once.

When both ears are stimulated, the response is considerably larger than that obtained from either alone (see fig. 2). In general, low intensity responses add almost perfectly. Higher intensity responses fail to yield the amplitudes expected from arithmetical addition of the two monaural effects (cf. Saul and Davis, 1932).

The mechanism of monaural masking has been discussed by Derbyshire and Davis (1935). The phenomenon was utilized in our earlier monaural

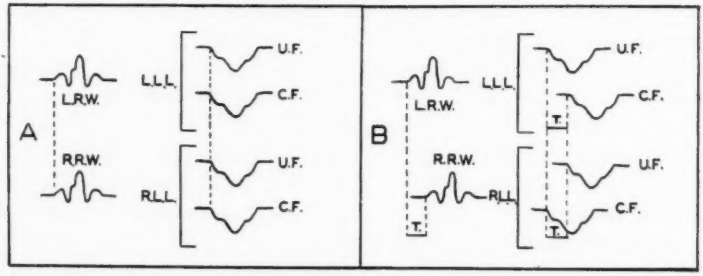

Fig. 1. Semi-diagrammatic representation of responses from the lateral lemniscus to (A) simultaneous and (B) non-simultaneous binaural stimulation. The stimuli were clicks at 60 per second. *L.R.W.* and *R.R.W.* stand for the responses recorded from the left and right round windows respectively. *L.L.L.* and *R.L.L.* stand for the responses recorded from the left and right lateral lemnisci respectively. *U.F.* and *C.F.* stand for the uncrossed and crossed fibers of the tracts respectively.

In A when the two ears are stimulated simultaneously, all responses recorded from both lateral lemnisci appear at the same minimum latency.

In B the right *R.W.* was stimulated after a time, *T*, had elapsed after the initiation of the *L.R.W.* response. In each lateral lemniscus the two responses can be recorded, separated by a time interval *T*, equal to the time of separation of the *R.W.* responses. This shows that whether the binaural stimulation be simultaneous or non-simultaneous, time relations are maintained precisely in each lateral lemniscus for both homolateral and contralateral components.

experiments as a means of analyzing complex wave forms. Since its physiological basis appears to be occlusion through competition for the same fibres by two stimuli arriving almost simultaneously, we should be able to test the possibility of convergence of the tracts from the two ears at the brain-stem level by sending the masking tone into one ear and the click into the other. Such binaural presentation did not bring about noticeable masking, even when the masking tone was twenty to twenty-five db higher in intensity than the click. This agrees with the results of Wegel and Lane (1924), who reported failure to obtain binaural masking in experiments with human subjects. This evidence argues against the

possibility of convergence of fibres from the two ears at the level of the cochlear nuclei or the superior olivary complex.

As would be expected from the observations on latency, the responses from crossed and uncrossed components of the lateral lemniscus are usually quite completely synchronized. This observation has been confirmed by recording from placements at which synchronous responses to tones are obtained for monaural stimulation. With such placements the responses for binaural stimulation are similar to those obtained for monaural stimulation but the amplitude is greater. In other words, the synchronous responses to tones obtained from the auditory tracts show the same kind of addition of amplitude as is found with clicks.

3. Non-simultaneous binaural stimulation. All the above observations were made with the stimuli to the two ears exactly simultaneous, as verified by the round window responses. When either stimulus is made to lead the other in time, a condition readily accomplished by changing the tube length on one side, both responses appear in the record from the lateral lemniscus with time differences preserved. (See fig. 1B.) If there were a high degree of occlusion, there should be a noticeable diminution of amplitude of the second response when it falls within the refractory period of fibres which have responded to the earlier stimulus. This was not observed.

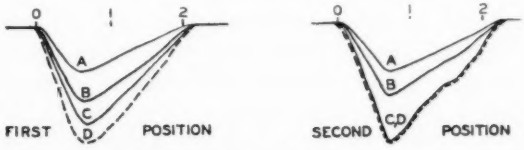

Fig. 2. Shows tracings made on the cathode-ray tube-face of responses of the lateral lemniscus to clicks at 60 per second at an intensity of −60 db. The figures indicate time in milliseconds from the beginning of the response.

In the first position, *A* is the response of the lateral lemniscus to stimulation of the left ear, and *B* to stimulation of the right ear alone. *C* is the response when both ears are stimulated simultaneously at the same intensity. *D* is the curve obtained by adding the ordinates of the two monaural responses. From this first position, then, there is an incomplete addition of the two monaural responses.

In the second position *A* and *B* are the responses of the lateral lemniscus to stimulation of left and right ears alone respectively. *C* is the response to binaural stimulation of the same intensity, and *D* is the curve obtained by adding ordinates of the two monaural responses. Here there is a perfect addition of the two monaural responses.

DISCUSSION. 1. *Similarity of responses from homolateral and contralateral tracts.* A. *Latencies.* We might expect to find a difference between the crossed and uncrossed latencies for, although synaptic delays should be of equal magnitude, conduction time would presumably be shorter for

the uncrossed tracts. We believe that our method is capable of detecting differences of latency of one-tenth of a millisecond. We really know very little about the total distance traversed by crossed and uncrossed responses from the cochlear nuclei to the lateral lemniscus. Certainly the acoustic striae deviate considerably from the shortest possible path. Decussation and redecussation may occur. However, the situation may be brought about, our observations point to equality of latencies and preservation of time differences in binaural effects.

B. *Thresholds.* Our observations on the equality of thresholds for crossed and uncrossed tracts is in agreement with the results of Mettler, Finch, Girden, and Culler (1934), who used the conditioned reflex method of testing hearing in animals before and after the auditory apparatus had been destroyed unilaterally.

2. *Interaction of pathways from the two ears.* There is apparently little convergence of the crossed and uncrossed fibres of the auditory tracts at this level. This might be expected from the data on binaural loudness in human subjects for, according to Fletcher and Munson (1933) and others, loudness is doubled when we compare listening with one ear to listening with two ears. Our failure to find a decrease in latency with simultaneous stimulation of the two ears, the lack of binaural masking, and equal amplitude of non-simultaneous responses stand as evidence which makes convergence and occlusion (central masking) unlikely. The evidence from masking is not conclusive, because ten per cent masking might escape detection. Any decrease in latency, however, would be immediately observable, and we can be reasonably sure that it does not occur. Imperfect addition of amplitude of responses of the two ears upon binaural stimulation might be taken to mean that approximately ten to twenty per cent of occlusion occurs within the range of intensities which we used. Uncertainty as to the exact physical behavior of concentric electrodes, however, makes us cautious in drawing any conclusions from amplitude measurements. Such objections do not apply to latency measurements.

The range of intensities over which it was possible to measure amplitude was of necessity quite short, i.e., 30 to 40 db from threshold. Above this intensity level the "unstimulated" ear shows a round window response when the opposite ear is stimulated. This type of error enters into all binaural experiments on human as well as on animal subjects, and must be taken into account in the interpretation of binaural hearing data. In our experiments, however, the threshold for "cross-stimulation" is probably lower than in human experimentation, due to the fact that here the bullae on both sides were opened.

3. *Auditory localization.* From experiments in human hearing, the localization of sound has been considered as primarily dependent on one

or more of three factors: 1, binaural differences in intensity; 2, binaural differences in phase, and 3, binaural differences in the time of arrival of the stimuli. According to our observations and those of Derbyshire and Davis (1935) phase and time differences must have a similar physiological effect, if we recall that stimulation occurs at a particular phase of the stimulating wave. In view of the decrease in latency with increasing intensity of stimulation reported in our earlier paper, greater intensity of stimulation at the ear nearer to the sound source would accentuate the time difference due to earlier arrival or earlier phase at this ear. We should expect, then, that intensity would play an especially important rôle in localization over the range of intensities below the level at which latency becomes a minimum. Above this intensity level, approximately 40 db above threshold, increasing intensity should have no appreciable effect on time of arrival of impulses at the "localizing center," although it would of course have an effect upon the total number of impulses arriving there. Over the low range of intensities, greater intensity at one ear should compensate for slight time advantage of the other ear in experimental situations.

Maintenance of rather exact time relations in the response pattern from the lateral lemniscus for frequencies lower than 500 to 750 cycles per second should be of importance for auditory localization and other binaural experiences, in so far as such phenomena depend upon phase differences at the two ears. Moreover, the abrupt upper limit of synchronization should be represented in some aspect of these phenomena. Thus, for example, Stevens and Sobel (1937) report that with 10 observers binaural beats, due to stimulation of the two ears by slightly different frequencies, could be observed only at frequencies below 750 to 800 cycles. The disappearance of binaural beats for higher frequencies may well be attributed to a failure of synchronization.

SUMMARY

Using cats as subjects, we recorded potentials between ground and one of three active regions: 1, the left round window; 2, the right round window, and 3, the lateral lemniscus. When the two ears were stimulated alternately by two carefully matched sound systems, we found that responses from the homolateral and contralateral components in the lateral lemniscus were practically equivalent in threshold, latency, and amplitude. Clicks and tones were used as stimuli.

When both ears were stimulated simultaneously (at intensities less than 40 db above the threshold for either ear), we found that 1, the binaural latency was not appreciably shorter than either monaural latency; 2, binaural masking was not demonstrable; 3, stimulation of both ears gave amplitudes expected from addition of the two monaural effects at low

intensity levels (less than 10–15 db above threshold), but not at higher intensities. Binaural responses to tones gave perfect synchronization of homolateral with contralateral fibres. These results generally argue against the convergence of the tracts from the two ears in the cochlear nuclei or superior olivary complex.

When one ear is stimulated slightly before the other, both responses appear in the record from the lateral lemniscus with the time difference preserved. The second response shows no diminution in amplitude.

These results are of significance for the problems of binaural interaction and auditory localization.

We wish to express our appreciation to Prof. Hallowell Davis for many valuable suggestions and for the facilities which he has placed at our disposal.

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THE RELATION BETWEEN BLOOD OSMOTIC PRESSURE, FLUID DISTRIBUTION AND VOLUNTARY WATER INTAKE

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The physiological basis of thirst has been a debatable subject for well over a century. Recent investigations have centered around the old controversy as to whether thirst is a general or localized sensation. The historical literature on this subject has been presented by Cannon (1918). Montgomery (1931) has briefly summarized the more recent contributions.

Schiff in 1867 (cited by Cannon, 1918) was of the opinion that thirst was a generalized sensation which arose from a lessened water content of the body. The local reference of the sensation of thirst to the pharynx he placed in the same category as the local reference of hunger to the stomach, or of sleepiness to the heaviness of the eyelids. Recent investigations, however, have stressed the relationship of water intake to the local conditions of the mouth especially with reference to salivary flow (Montgomery, 1931).

The influence of general body hydration and the distribution of body fluids upon thirst has received scant attention. In the present study, observations on voluntary water intake were correlated with changes in blood osmotic pressure and the general distribution of the body fluids.

PROCEDURE. Dogs were used as the experimental animals. A normal blood sample was taken from the femoral artery, after which the animals received, intravenously, 2.5 cc./kilo of either 20 per cent NaCl or 40 per cent urea. These concentrations are approximately isosmolar. Thirty minutes were allowed to elapse for the complete equilibration of the substances injected during which time the dogs were kept quietly on the table and were not given access to water. At the end of this period a second arterial blood sample was procured and the animals then returned to their cages where water was available ad libitum. Their fluid intake was recorded at fifteen minute intervals. Blood samples were obtained at intervals over six hours.

Measurements were made on the serum of total osmotic pressure by the vapor pressure method of Hill (1930) and specific gravity by the falling drop method of Barbour and Hamilton (1926).

EXPERIMENTAL RESULTS. *Osmotic pressure.* A marked increase in the blood osmotic pressure resulted from the injection of hypertonic urea and NaCl. Inasmuch as the solutions injected were approximately isosmolar and given in proportion to body weight, the increases in osmotic pressure in all experiments were almost identical (table 1). It is of interest in view of the constancy of the osmotic pressure rise to calculate the per cent of active body water from these figures. The normal osmotic pressure of the dogs receiving NaCl expressed in terms of the concentration of an isosmolar NaCl solution averaged 152.1 m. eq. per liter. After injection this rose to 164.6 m. eq. per liter, an increase of 12.5 m. eq. per liter. This resulted from an injection of 2.5 cc. per kilo of 20 per cent NaCl or 8.55

TABLE 1

Serum osmotic pressure and specific gravity changes following the intravenous injection of 2.5 cc. per kilo of 20 per cent NaCl and of 40 per cent urea

DOG	INJECTION	SERUM O. P.*		SERUM S. G.	
		Initial	30 minutes after injection	Initial	30 minutes after injection
A	20% NaCl	0.902	0.980	1.0228	1.0197
	40% Urea	0.871	0.947	1.0201	1.0202
B	20% NaCl	0.897	0.966	1.0216	1.0188
	40% Urea	0.892	0.971	1.0209	1.0212
C	20% NaCl	0.873	0.942	1.0212	1.0196
	40% Urea	0.873	0.946	1.0217	1.0220
D	20% NaCl	0.880	0.955	1.0213	1.0190
	40% Urea	0.901	0.982	1.0210	1.0215

* Serum osmotic pressure is expressed as per cent concentration of an isosmolar NaCl solution.

m. eq. per kilo. Thus for every kilo of body weight the animals received 8.55 m. eq. of NaCl and for every liter of body water they gained 12.5 m. eq. Despite the fact that the Na and Cl remain in the extracellular fluid, all of the body fluids contribute to the osmotic equilibration. Therefore we may say that the active water in the body may be considered $\frac{8.55}{12.5}$ or 68 per cent. Using this same type of calculation for the urea experiments, a value of 65 per cent is obtained. This is in close agreement with the accepted figures obtained by other methods of calculation. It is subject to error due to the renal activity during the thirty minute equilibration period.

Specific gravity. Although the blood osmotic changes in both the urea and NaCl series were identical, there were marked differences observed in the specific gravities. The injection of the hypertonic NaCl was accompanied by a reduction in serum specific gravity, whereas no demonstrable change followed the administration of urea. Inasmuch as serum specific gravity is essentially a reflection of the concentration of serum protein, this reduction after saline administration is evidence of a shift of water from the cells to the extracellular reservoir due to the impermeability of the cell membrane to the ions involved. After the injection of a permeable molecule such as urea, on the other hand, the specific gravity remained unchanged. Thus hypertonic urea contributed to the high total osmotic pressure of the blood without any shifts between intra and extracellular water. In short these injections resulted in two groups of animals, both characterized by abnormally high osmotic pressures of the same degree. In one group (NaCl) the increase in cellular osmotic pressure was accompanied by a loss of fluid, in the other (urea) the increase in cellular osmotic pressure resulted from the increased concentration of a freely permeable molecule with no fluid loss. It should be emphasized that the osmotic pressure within the cells of both groups of animals should be the same despite the difference in fluid distribution.

Water intake. Despite the identical rise in osmotic pressure, the water intake of these two groups differed markedly. The average voluntary consumption during the first fifteen minutes, of those animals receiving urea, was 12 cc. per kilo, whereas during the same period those dogs which had received NaCl drank 32 cc. per kilo. At the end of one hour the average intakes had risen to 20 cc. per kilo and 42 cc. per kilo respectively.

As would be expected from the different levels of voluntary fluid intake, there was a pronounced difference between the two groups in the return of the blood osmotic pressure to normal. Figure 1 depicts the complete protocol of a typical experiment in which dog B received injections of NaCl and urea seven days apart. Although the respective increases in osmotic pressure were identical, the fluid intake following NaCl was over twice that following urea. As a result the blood osmotic pressure after the administration of NaCl returned to its normal level within an hour. On the other hand, although there was some evidence of thirst after the urea administration, the voluntary water consumption was not sufficient to lower the osmotic pressure of the blood to its original level and the animal seemed comfortable, insofar as the sensation of thirst was concerned, with a blood osmotic pressure appreciably higher than normal. The responsibility of the return to a normal value was seemingly left to the kidney.

It is of interest to observe that the serum of the animal depicted in figure 1, before receiving NaCl was isosmolar with a 0.897 per cent NaCl solution. The dog received 28.5 cc. of 20 per cent NaCl. In order to render

this isotonic the animal would have to drink $28.5 \times \frac{20}{0.897}$ or 635 cc. of water. The animal actually consumed during the first hour 610 cc. which,

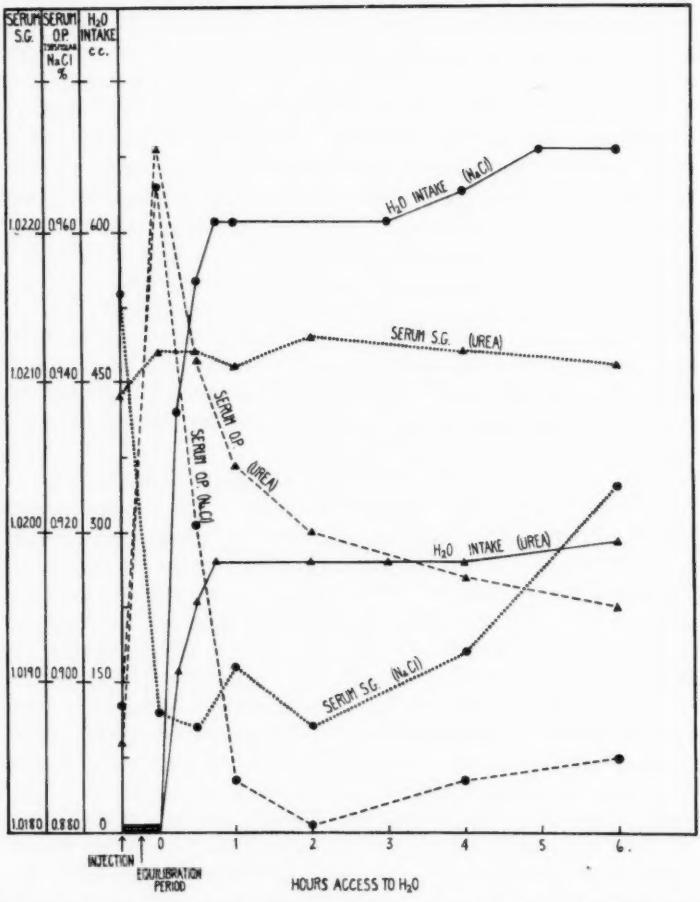

Fig. 1. Serum osmotic pressure and specific gravity changes, and voluntary water intake in dogs following the intravenous injection of 2.5 cc. per kilo of isosmolar hypertonic NaCl (20 per cent) and urea (40 per cent).

plus the "osmotic work" of the kidney, was more than sufficient to dilute the blood to its original level.

Discussion. It can be seen from the above results that the sensation of thirst depends upon more than a mere increase in the total osmotic

pressure of the body fluids. After the administration of hypertonic NaCl and urea the increases in blood osmotic pressure were the same. The essential difference between the two groups of experiments was in the water content of the cell. The logical conclusion to draw from the above results is that *cellular* dehydration rather than an increase in cellular osmotic pressure *per se* is the stimulus of true thirst.

It is of interest in this regard to cite some observations on dogs with abnormal water distribution characterized by anhydremia associated with a lowered blood osmotic pressure and cellular hydration. It has been shown by Darrow and Yannett (1935) that when large amounts of extracellular electrolyte, unaccompanied by H_2O are withdrawn from animals, the selective loss of extracellular ions results in a shift of fluid into the cells in order to maintain osmotic equilibrium. Such animals show anhydremia characterized by increased concentration of blood hemoglobin, specific gravity, hematocrit, and serum protein, accompanied by a cellular hydration. While utilizing dogs in this condition for other observations (Gilman, 1934) it was observed that in the acute stage of anhydremia such animals shunned water. Seemingly, despite the anhydremia and the striking dry appearance of the oral mucous membranes and the probable lack of salivary secretions, these dogs did not experience the sensation of thirst. It is tempting to attribute this to the low electrolyte and high water content of the cells.

The differences in voluntary water intake following the administration of NaCl and urea is of fundamental significance. Gamble and co-workers (1929, 1934) have emphasized "An economy of water in renal function referable to urea." In their experiments the urinary excretion of urea and electrolytes was studied in rats receiving varying proportions of these substances in the diet. The animals were allowed water ad libitum. It was found that the water volume necessary for the excretion of varying proportions of salts and urea were not additive but that there was an economy of water in renal function referable to the excretion of urea. Reference to Gamble's data, however, shows that urine output varies with water consumption, and it is logical to assume that thirst was the primary mechanism involved in this economy. Thus the animals always drank sufficient fluid to compensate for the salt intake to the isotonic level. Urea added to the diet seemingly did not increase the thirst stimulus until its osmotic concentration was greater than that of the salt.

SUMMARY AND CONCLUSIONS

Voluntary water intake in dogs differed markedly following the intravenous injection of hypertonic solutions of isosmolar NaCl and urea despite identical increases in blood osmotic pressure. After the administration of hypertonic NaCl, animals immediately consumed sufficient

water to dilute their blood to its preinjection level, whereas after urea, in the absence of a thirst stimulus, the osmotic pressure of the blood remained elevated. This difference in water intake was attributed to the differences in the water content of the cell. Experiments were cited in which marked anhydremia accompanied by cellular hydration failed to stimulate thirst.

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EFFECT OF POSTURE ON CARDIAC OUTPUT

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The balance of evidence as obtained by various investigators, employing different methods, indicates that cardiac output is smaller in the sitting and standing positions than in the recumbent position. Recent investigations (1–5) in which the Grollman acetylene method was used have given results in the same direction. Grollman (6) claims, however, that cardiac output does not vary with posture, a conclusion supported by Bielschowsky (7) who used the acetylene method. These discordant results obtained by the same method, augmented by Gladstone's criticism and modification of the Grollman acetylene method (8) prompted this reinvestigation of the problem.

We have used the original Grollman acetylene method (9) and the Gladstone modification (8) in a total of over 200 determinations on 5 subjects each studied at intervals over periods of 4 to 6 months. The subjects were healthy adult males ranging in ages from 21 to 36 years, three were members of the staff and two were medical students. Each subject was thoroughly familiar with the various procedures and was always studied under basal conditions. The experimental routine was standardized as much as was possible. The subject arrived at the laboratory as early in the morning as was convenient and reclined on a comfortable cot for at least a half hour. At the end of the rest period, the oxygen consumption was measured in a Benedict-Roth recording spirometer, during which period the room temperature (wet and dry) and the barometric pressure were noted. The cardiac output was determined five minutes after the completion of the oxygen consumption test. Pulse rate, blood pressure and body temperature were determined at intervals during the experiment. In order to avoid the possibility of the rest period in the recumbent position affecting the final comparative results, a few experiments were carried out with the subject sitting quietly in a straight-backed chair with feet on the floor for the preliminary rest period. After the determinations were made in the recumbent or sitting position, they were repeated in another position, the change being made by the subject with as little expenditure of energy as possible. After maintaining the new position for at least 5

minutes, O_2 consumption was again measured and after about 5 more minutes, a second cardiac output was determined. On some days, the subject moved to a third position or returned to the original position in which the determinations were again repeated. On most days, however, determinations were made in only the recumbent and standing positions. When the subjects were in the erect position, they were "at ease" leaning lightly against the wall to prevent swaying.

The results are given in tables 1 to 3. The averages in table 1 are derived from experiments in which two or three positions were studied by one method on the same day and those in which both methods were used for one or two positions on the same day. The values for subject H. S. M.

TABLE 1

Comparison of Grollman and Gladstone methods

Averages of determinations

SUBJECT	Method	RECUMBENT				SITTING				STANDING			
		Number of determinations	O_2 consumption	A-V O_2 difference	Cardiac output	Number of determinations	O_2 consumption	A-V O_2 difference	Cardiac output	Number of determinations	O_2 consumption	A-V O_2 difference	Cardiac output
			cc. per min.	*cc. per l.*	*l. per min.*		*cc. per min.*	*cc. per l.*	*l. per min.*		*cc. per min.*	*cc. per l.*	*l. per min.*
P. C. F.	Gladstone	9	205.3	57.3	3.58	4	202.9	66.3	3.06	12	227.1	75.0	3.03
	Grollman	11	206.6	58.3	3.54	8	211.2	73.0	2.89	12	229.0	86.0	2.66
	Percent difference				1.1				5.5				12.2
H. S. M.	Gladstone	4	235.1	43.9	5.36					4	247.1	59.6	4.15
	Grollman	4	235.1	47.4	4.96					4	247.1	79.9	3.10
	Percent difference				7.5								25.3

involve only the latter type of experiment. Table 2 gives the average values of all determinations in different positions obtained with the Gladstone method. Table 3 includes only those values in which determinations were done by the Gladstone method in both positions on the same day (paired determinations). An examination of these tables shows that the output per minute and per beat in the standing is definitely less than in the recumbent position regardless of the method used. The decrease in output is directly attributable to the relatively greater increase in A-V O_2 difference than in O_2 consumption, as particularly well illustrated in the case of H. S. M. whose O_2 consumption was approximately the same in the recumbent and standing positions and who consistently showed an

increase in the A-V O_2 difference of between 30 to 50 per cent on standing. The same results are obtained irrespective of the sequence in which the subjects assume the respective positions.

TABLE 2

Averages of all determinations

Gladstone method

SUBJECT	RECUMBENT						STANDING					
	Number of determinations	O_2 consumption	A-V O_2 difference	Cardiac output	Stroke output	Cardiac index	Number of determinations	O_2 consumption	A-V O_2 difference	Cardiac output	Stroke output	Cardiac index
		cc. per min.	*cc. per l.*	*l. per min.*	*cc. per beat*			*cc. per min.*	*cc. per l.*	*l. per min.*	*cc. per beat*	
P. C. F.	9	205.3	57.3	3.58	57.7	2.04	12	227.1	74.9	3.03	37.3	1.77
H. S. M.	17	227.8	48.3	4.72	79.9	2.57	10	232.0	64.6	3.60	44.0	1.98
R. E. M.	13	229.5	52.1	4.41	67.7	2.51	8	242.6	59.2	4.10	47.5	2.38
J. W. H.	9	197.4	38.1	5.19	91.9	3.03	16	223.5	58.3	3.83	41.3	2.23
L. A. T.	12	236.8	42.1	5.63	109.1	2.98	12	289.8	58.3	5.00	61.9	2.69
Average..		219.4	47.6	4.61	81.3	2.63		243.0	63.1	3.85	48.0	2.21
Per cent deviation from recumbent values.....								+10.8	+32.6	−16.5	−40.9	−16.0

TABLE 3

Averages of paired determinations

Gladstone method

SUBJECT	RECUMBENT						STANDING				
	Number of pairs	O_2 consumption	A-V O_2 difference	Cardiac output	Stroke output	Cardiac index	O_2 consumption	A-V O_2 difference	Cardiac output	Stroke output	Cardiac index
		cc. per min.	*cc. per l.*	*l. per min.*	*cc. per beat*		*cc. per min.*	*cc. per l.*	*l. per min.*	*cc. per beat*	
P. C. F.	8	210.9	57.4	3.67	59.3	2.12	228.2	76.7	3.00	36.1	1.75
H. S. M.	2	217.4	42.8	5.08	80.9	2.72	215.7	67.7	3.19	36.3	1.71
R. E. M.	4	221.3	46.9	4.72	75.4	2.70	235.8	55.8	4.22	48.3	2.49
J. W. H.	6	196.3	34.4	5.74	99.8	3.37	244.0	61.7	3.95	44.2	2.32
L. A. T.	8	239.9	40.3	5.95	116.8	3.17	285.4	54.6	5.23	66.5	2.85
Average..		217.2	44.4	4.90	86.4	2.82	241.8	63.3	3.82	46.3	2.22
Per cent deviation from recumbent values....							+11.3	+42.6	−22.0	−46.4	−21.3

Hamilton, Spradlin and Saam (10) and Starr and Collins (11) pointed out that recirculation constitutes a possible source of error in the Groll-

man method. One of the fundamental assumptions of the method is that the blood reaching the lungs during the test period contains little or no blood which has already been exposed to the acetylene, since such recirculated blood, because of its acetylene content, would not take up the requisite amount of acetylene from the lungs. This would result in a low acetylene difference, a high A-V O_2 difference and a low output. Gladstone (8) has recently measured the rate of acetylene diffusion from the lungs to the blood during the progress of the rebreathing and showed that in the sitting and semi-recumbent positions, the diffusion rate of acetylene markedly decreased at about 11 seconds. We have done similar experiments on all of our subjects by analyzing six samples, collected at intervals of two seconds or more during the rebreathing, plotting the logarithms of the C_2H_2 concentration against time and calculating the A-V O_2 difference for each interval. The rate of diffusion of acetylene from the lungs to the blood should be proportional to the progressively decreasing alveolar concentration of that gas. The latter concentration charted as ordinate against time as abscissa should follow an exponential curve and the logarithms of the concentration should follow a straight line providing the blood flow through the lungs is constant and there is no recirculation during the rebreathing procedure. A-V O_2 differences calculated by pairing any two samples should agree reasonably well with each other. Curves plotted from data of 13 such experiments with the subjects in the recumbent position show uniform slopes (indicative of a uniform C_2H_2 diffusion and thus no recirculation) for at least 20 to 23 seconds and the A-V O_2 differences are at approximately the same level throughout the rebreathing period (fig. 1, recumbent). The same number of experiments done with the subjects in the standing and three in the sitting position, however, show a flattening of the curves at from 7 to 14 (average 10.2) seconds and at from 8.2 to 11.7 (average 10.5) seconds, respectively. Calculation and comparison of the natural tangents corresponding to the angles formed by the first and second portions of the curves show an average change in slope of 54 per cent in the standing and 52.3 per cent in the sitting position. The A-V O_2 difference increases abruptly at the same time as the slope changes and then usually shows a progressive rise (fig. 1, standing). In some cases a plateau is maintained, which, when present, must arise from a cancellation of errors. The hyperpneic rebreathing accelerates the blood flow to the lungs, thus increasing the O_2 content of the returning mixed venous blood and decreasing the O_2 difference in the two samples which tends to compensate for the error in the acetylene difference.

The above results account for the close agreement of the determinations made by the two methods in the recumbent position and the difference in the standing and sitting positions (table 1). Since the Grollman rebreathing procedure must be prolonged for 15 to 18 seconds in order to obtain

good mixing before the first sample is drawn, the values for A-V O_2 difference in the sitting and standing positions are high and the corresponding outputs low because of recirculation setting in during the rebreathing procedure. In the Gladstone method, the entire rebreathing procedure is restricted to less than 10 seconds and the values obtained should, therefore, be accurate providing adequate mixing can be attained in the short interval (4–5 sec.) allowed before drawing the first sample. We have confirmed Gladstone's (8) contention that homogeneity of gases is obtained by drawing first samples simultaneously from the mouth and distal ends

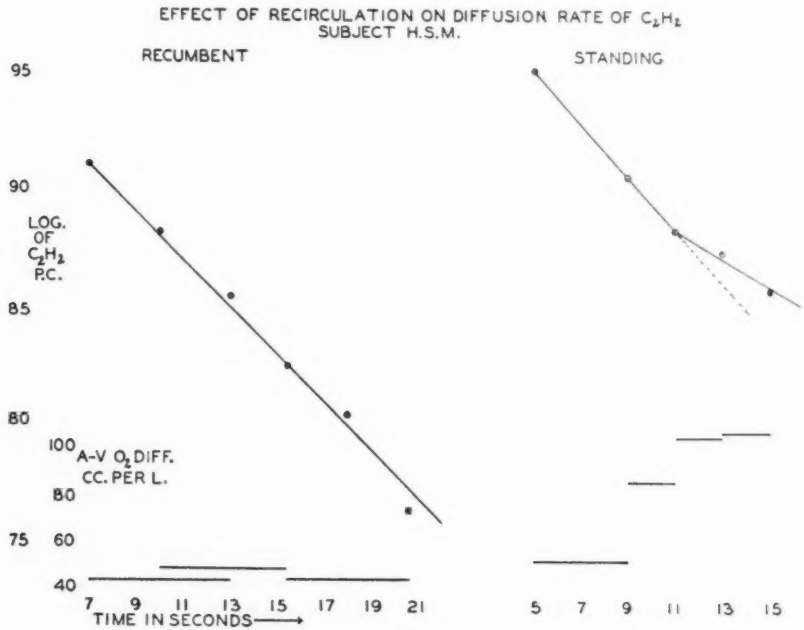

Fig. 1

of the rebreathing bag and pairing each with a single second sample for calculating A-V O_2 difference. He obtained values for samples thus collected which agreed within 5 per cent. Similar experiments in our hands yielded an average error of about 8 per cent, the mouth sample which remains slightly longer in the lungs than the distal sample usually showing more CO_2 and less O_2 and C_2H_2 than the distal sample. A further check on the adequacy of mixing in the shortened rebreathing procedure is, we believe, given by the results of the six-sample experiments mentioned above. The close agreement of the A-V O_2 difference when any two of

the samples (taken before recirculation in the standing and sitting positions) are paired must mean that homogeneity was obtained before the first sample was drawn.

We are, at present, unable to explain the more rapid recirculation in the sitting and standing positions as compared with recumbency. It is conceivable that the former positions facilitate the return of a disproportionately large amount of blood from the short systemic circuits although the amount from the extremities and splanchnic area is decreased. That these short systemic paths carry appreciable quantities of blood is evidenced by the measurements of Starr and Collins (11) who showed that in 15 seconds 44 per cent of the cardiac output of the dog had passed through the systemic paths and back to the heart.

While our results indicate the unreliability of the Grollman rebreathing procedure for the sitting and standing positions, they emphasize its validity in measuring outputs in the recumbent position. In spite of the marked differences in the rebreathing procedures, not only do the recumbent average values obtained by the two methods agree closely (table 1) but the variation in the individual values for a period of 4 months is of the same character and magnitude (less than 10 per cent from the mean). Furthermore, the average of 5 determinations on subject P. C. F. made in the recumbent position by the Grollman method 4 years previously is within 2 per cent of the present average value. Such duplication of results can hardly be fortuitous.

On the other hand, greater variation is found in the standing (and sitting) position even when the Gladstone method is used. This is to be expected because of the greater difficulty in exactly duplicating the posture on different days and because of the varying ability of different individuals to maintain the erect posture for even short periods of time. Three of our subjects (P. C. F., H. S. M., R. E. M.) found no difficulty in standing quietly. P. C. F. and H. S. M. usually showed no significant change in systolic and a rise of 5 to 10 mm. Hg in the diastolic pressure over their recumbent values. R. E. M. usually showed a drop of 5 to 10 mm. Hg in the systolic but no significant change in the diastolic pressure. The pulse pressures in these individuals on standing varied from 22 to 34 mm. Hg. J. W. H. and L. A. T., however, tended to develop vertigo and to faint. The length of time they were comfortably able to stand varied. On some days they experienced no discomfort on standing as long as 45 minutes, on other days fainting intervened from 10 to 25 minutes after assuming the erect position. Their usual response on standing for about 20 minutes was a decrease of about 10 mm. in systolic pressure accompanied by a rise of 5 to 10 mm. in diastolic pressure, resulting in considerable narrowing of the pulse pressure to as low as 14 and 12 mm. in several instances. Just before fainting supervened there was a rapid fall of both systolic and diastolic pressures. Fainting sensations and vertigo usually

followed when the pulse pressure decreased below 20 mm. Hg (4). No correlation could be found between the size of the output per minute or per beat and the ability to stand.

Our results thus far fail to show any correlation between cardiac output and body temperature, barometric pressure, room temperature or relative humidity. Scott (5) found that during the winter season in the standing position high A-V O_2 differences were usually associated with high O_2 consumption, while in the summer this relationship was reversed. Our data indicate a possible tendency in this direction.

SUMMARY

The original Grollman acetylene and the modified method of Gladstone have been used to study the effects of posture on the cardiac outputs of 5 experienced subjects. Determinations made at intervals over periods of 4 to 6 months show a consistent decrease in output on quiet standing as contrasted to recumbency. The average change per subject with the Gladstone method ranged from 5 to 26 per cent for all determinations and 8 to 36 per cent for paired observations. Greater differences are obtained when the Grollman method is used.

Measurement of the acetylene diffusion during the rebreathing procedure gives no evidence of recirculation occurring in the recumbent position within 23 seconds. In the standing and sitting positions, however, acetylene diffusion is retarded after about 10 seconds, indicative of recirculation. Adequate mixing can be obtained by the Gladstone rebreathing procedure within 4 to 5 seconds and consistent results can be obtained by use of this method in the standing and sitting positions. Both methods give similar results in the recumbent position.

We are grateful to Prof. Henry Laurens for his suggestions and interest in this investigation and to Dr. P. C. Foster for his assistance in the early part of the work.

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SECRETIN IS A TRUE CHOLAGOGUE

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Mellanby (1) failed to obtain an increase in bile formation on the injection of secretin after obstruction of the pancreatic vein in the cat. This observation led him to believe that the cholagogue effect of secretin is secondary to its action on the pancreas. Still, McBean and Ries (2) removed the intestine, spleen and pancreas and tied the gastric blood vessels in the dog and obtained an increase in bile flow on injecting secretin. Since they did not state whether the cystic duct had been occluded in their experiments and their results were contrary to those of Mellanby, and since it is difficult for us to believe that a species difference in this regard exists, we have reinvestigated the problem.

METHODS. Dogs and cats anesthetized with pentobarbital were used. The cystic duct was tied; the common duct was cannulated; and the carotid artery was connected to a mercury manometer. A control rate of bile flow was recorded. Then the entire gastro-intestinal tract from the cardiac orifice to the rectum and the spleen and pancreas were removed, an operation which in these experiments did not depress blood pressure more than 30 mm. Hg. The liver now received blood only through the hepatic artery. A control flow of bile was recorded and secretin injected.

The secretin used in these experiments was prepared by treatment with butyl alcohol of a secretin preparation used previously (3) and forms a crystalline, active compound with picrolonic acid. The average threshold dose for the pancreas in the dog is 0.015 mgm., 0.24 mgm. in the dog causing the production of about 5.5 cc. (average) of pancreatic juice. It contained no cholecystokinin, at least in the doses used. The dogs were given sixteen pancreatic secretory threshold doses and the cats (3–4.5 kilos) eight doses.

RESULTS. In the first dog the removal of all the abdominal viscera except the liver and urinary bladder resulted in an almost complete suppression of bile flow. The blood pressure after the operation was higher than before. In the other three the suppression of bile flow was not so

[1] Becado. Comision Nacional de Cultura, Buenos Aires.

marked. As will be seen from the inspection of table 1, the injection of secretin increased the flow of bile in all of the nine tests on four dogs.

In the first cat the removal of the abdominal viscera except the liver and urinary bladder resulted in a complete suppression of bile flow. The blood pressure was not changed by the operation. The liver failed to respond to secretin and even to bile salts. A careful dissection indicated that the hepatic artery may have been kinked by the ligature on the pan-

TABLE 1

The effect of secretin on bile formation after removal of the gastro-intestinal tract, spleen and pancreas

ANIMAL	BILE COLLECTED IN CUBIC CENTIMETER IN HALF HOUR			MAXIMUM RATE OF FLOW PER HALF-HOUR AFTER SECRETIN
	Control before operation	Control after operation	Secretin effect after operation	
	cc.	cc.	cc.	
Dog 1	1 2	0 05	0 20	1 2
		0 05	0 65	2 5
Dog 2	2 1	1 0	1 50	3 0
		0 75	1 25	2 6
		0 4	1 5	2 5
Dog 3	1 0	0 60	0 75	2 25
		0 6	0 80	2 25
Dog 4	3 6	0 75	1 40	1 8
		0 75	1 50	2 1
Cat 1*		0	0	0
Cat 2	1 80	1 10	1 6	1 0
Cat 3	0 9	0 6	1 10	1 1
		0 6	1 0	1 8

* This cat would not secrete bile when bile salts were injected.

creato-duodenal blood vessels. The data in table 1 and the tracing in figure 1 show that secretin increases the flow of bile after the liver of the cat has been completely deprived of portal blood supply.

It was found that 0.12 mgm. of secretin in the "eviscerated" cat caused the production of about as much bile as 0.50 mgm. of sodium dehydrocholate. The volume of bile produced in response to secretin amounts to only from one-tenth to one-fifth that of the volume of pancreatic juice secreted.

Discussion. We have no way of accounting for the negative result obtained by Mellanby in his apparently single experiment in the cat. He does not present data showing a control flow of bile after ligating the pancreatic vein and prior to the injection of secretin, which in his experiment did not increase the flow of bile above the control flow of the pre-ligation period.

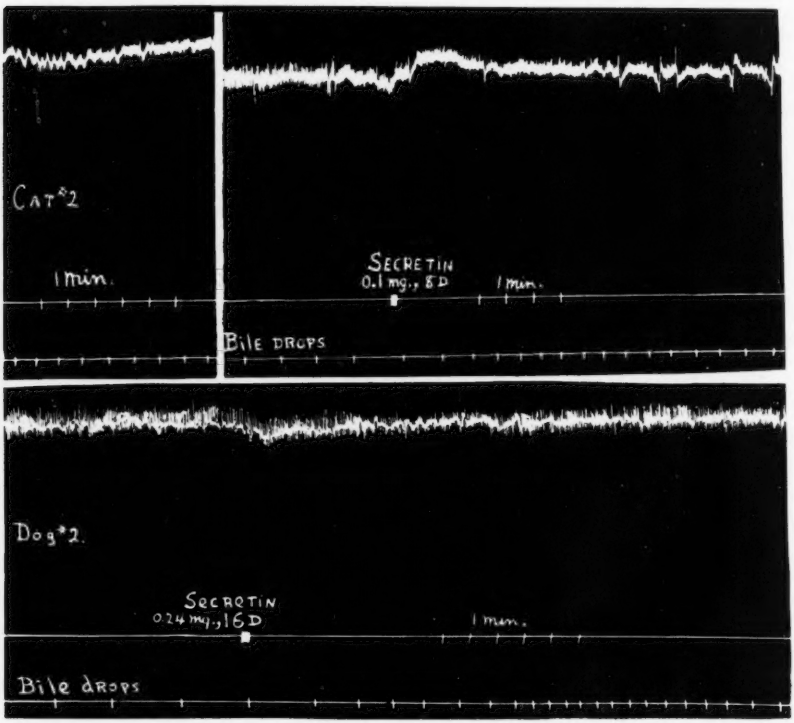

Fig. 1. The first portion of the upper tracing was obtained on cat 2 before the removal of the gastro-intestinal tract, pancreas and spleen. The second portion was obtained after evisceration. The short latent period of the hepatic response to secretin is quite evident. The lower tracing shows the response of an "eviscerated" dog to secretin. The short latent period is also evident.

The character of the biliary response to secretin, we believe, precludes the possibility of the increase in flow being due to a mechanical effect on the bile ducts. This possibility has been adequately excluded by the experiments of both Mellanby and Still.

Our observations indicate that removal of the gastro-intestinal tract,

pancreas and spleen for some reason shortens the latent period of the biliary response to secretin. In the intact animal the latent period of the biliary response varies from 6 to 10 minutes. After removal of the gastrointestinal tract, etc., the latent period observed varied from three to six minutes. The latent period of the pancreatic response varies from 1 to 3 minutes usually. It should be further noted, as is agreed by all investigators in this field, that secretin produces a considerably more copious flow of pancreatic juice than bile.

CONCLUSION

After the removal of the gastrointestinal tract from esophagus to rectum, the spleen and the pancreas in the cat and dog, secretin increases the formation of bile. This observation, along with corollary evidence submitted by Mellanby and Still, warrants the conclusion that secretin is a true cholagogue.

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REFLEX INHIBITION OF THE KNEE-JERK FROM INTESTINAL ORGANS[1]

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During an investigation on the influence of cortical stimulation upon the knee-jerk of the narcotized monkey the rhythmically elicited knee reflexes became gradually weaker for no apparent reason. Suddenly the animal voided a large quantum of urine, after which the reflexes returned to their previous size. Later on, when the reflexes diminished again, expression of the urinary bladder resulted in prompt and lasting enhancement of the knee-jerks.

Various reflexes from the urinary bladder and other abdominal organs upon the skeletal musculature are well known (Carlson and Luckhardt, 1905; Head and Riddoch, 1918; Miller, 1924; Danielopolu, 1927). Inhibition of the knee-jerk upon electrical stimulation of the central end of the severed vagus has been observed by King, Blair and Garrey (1916), Schweitzer and Wright (1936), but that distention of the bladder would inhibit the knee-jerks was unknown to us and we have been unable to find any reference in the literature to this effect. Therefore, this problem was investigated in some detail. It was found that distention of the urinary bladder, the gut or the gallbladder invariably results in a marked diminution or in disappearance of the knee-jerks. During such distention the knee-jerks are partially or totally inhibited.

METHODS. The animals used were Macaca mulatta (rhesus) monkeys and cats. All animals were fully anesthetized with Dial (Ciba),[2] 0.45 cc. per kilogram body weight for the monkey, 0.55 cc. per kilo for the cat, half of the dose being given intraperitoneally and half of it intramuscularly. This procedure results in an excellent, even anesthesia, lasting for many hours.

The knee reflexes were elicited every two to three seconds by a tap on the patellar tendon. To ensure equal mechanical stimulation, equal intervals and easy adjustment of the strength of stimulation a mechanical

[1] The costs of this investigation were defrayed by a grant from the Fluid Research Fund of the Yale School of Medicine.

[2] The Dial was kindly put at our disposal by Doctor Haskell of the Ciba Co., to whom we wish to tender our sincere thanks.

device was used (see fig. 1a). The animal was put in the prone or supine position on the operating-table near one edge, its head fixed in a head-clamp, its front legs tied down. The hind leg of the animal near the edge of the table was always used for recording the knee-jerks and was fixed by drills screwed into the greater trochanter and the external epicondyle of the femur. The knee was kept in a flexed position by the pull of a rubber band attached to the ankle and one of the stands on the table, thus keeping the extensors of the knee under an appropriate, easily adjustable tension. It was found that the correct amount of tension on the stretch-muscle of the knee is a very important factor for optimal knee-jerks in the monkey.

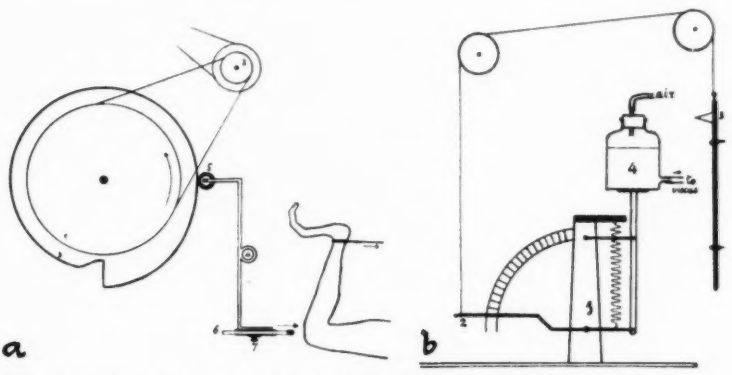

Fig. 1. a. Apparatus used for rhythmically eliciting knee-jerks. *1* and *2* = motor-driven pulleys. *3* = large wooden disk with a notch, along the circumference of which rolls one arm of lever, *5*. Everytime *5* falls into the notch, when *3* rotates with *2* and *1*, the pencil *6* hits with its eraser the patellar tendon of the animal. Set screw *7* allows adjustment of the strength of the tap.

b. Apparatus to record volume of water with which viscus is distended. See text.

The distention of the viscus under experimentation was effected by forcing water into it. A wide glass cannula was inserted into the fundus of the urinary bladder, into a tied-off loop of the ileum, or into the fundus of the gallbladder. In the experiments on the urinary bladder the urethra was clamped off to prevent escape of fluid; in those on the gall bladder the ductus choledochus was not ligated, so as not to interfere with the nerve supply to the gallbladder. The water was introduced into the viscus under experimentation from a Mariotte bottle, standing on a postal spring balance. At the beginning of an experiment the bottle was always filled until the surface of the water was at the level of the animal's abdomen. When the fluid is expelled from the bottle into the viscus by air pressure, the weight of the bottle decreases and the pointer

of the balance goes down. On release of the pressure all or part of the fluid returns to the bottle, the weight of which increases correspondingly and makes the pointer of the balance rise again. These movements of the pointer were recorded with a vertically moving writing point on the smoked paper of a kymograph (see fig. 1b). The pressure under which the water distends the viscus was recorded on the paper with a mercury manometer underneath the volume-record. Below this record the knee-jerks were recorded isotonically with two Marey-tambours.

To obtain greatest regularity of the knee-jerks it is necessary to reduce extraneous stimuli, except for the blow on the patellar tendon, as much as possible. In the beginning of the first experiment the knee reflexes showed great irregularity but became regular in size as soon as the stand of the tendon-hammer was taken away from the operating-table, on which the animal was lying, and fixed to the table on which the motor and rotating disk were mounted. Apparently the vibration of the table with each blow upset the spinal mechanisms. This susceptibility to vibration is still more clearly brought out by the fact that tapping of the operating-table invariably results in a marked reduction and often in an obliteration of the knee-jerks.

It was found also that the regularity of the knee reflexes was greatly enhanced if the weight of the hindpart of the body (with the animal in the prone position) was not carried exclusively by the two screws in the femur, but partly also by a sling around the other thigh, suspended from a supporting rod.

Results. The distention of the urinary bladder of the monkey invariably results in a marked diminution of the knee-jerks (see fig. 2_1). The position of the animal also has an influence, the inhibition being more pronounced when the animal is lying in the prone position, less in the supine position. A similar inhibition of the knee-jerks is evidenced upon distention of the gallbladder or a short loop of the ileum. See figures 2_2 and 2_3.

The latency with which the diminution of the knee-jerks sets in differs in the various animals. Usually it is short, the first knee-jerk after the beginning of the distention being already markedly decreased; sometimes the first 3 to 5 knee-jerks after the onset of the distention become smaller and smaller. It seems as if the inhibition is more abrupt in those animals in which the wall of the abdominal organ does not readily yield to the pressure. The return of the knee-jerks to their previous size, when the distention is released, differs also in various animals. In some of them it is abrupt, in other animals gradual. Sometimes the knee-jerks are temporarily larger than before the distention.

In one monkey, which was examined in the supine position, it was observed that each tap on the patellar tendon was accompanied not only by a knee-jerk, but also by a crossed adductor reflex. During distention of the ileum the knee-jerk was completely inhibited, the crossed adductor reflex remained, apparently unchanged.

Inhibition of the knee-jerk by distention of the bladder, the gut or the

gallbladder is also observable in the cat, but the effect is much less marked than in the monkey.

This inhibition is a spinal reflex, as is evidenced by the fact that it is still present in the spinal monkey, with transection of the cord in the mid-thoracic region. As the knee-jerks are absent for a few days after such transection in the monkey, one has to wait until these reflexes have reappeared, which usually occurs after two to five days. Figures 3_1 and 3_2 demonstrate the presence of inhibition of the knee-jerk in the spinal monkey upon distention of the urinary bladder and a loop of the ileum. This finding does not preclude possible influence from higher levels of the central nervous system on this phenomenon, but shows conclusively that it is primarily spinal in origin.

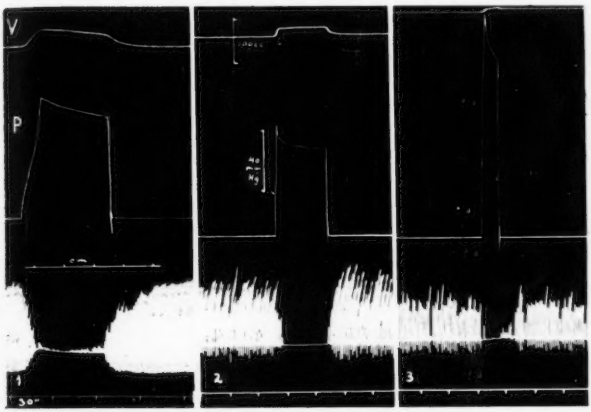

Fig. 2

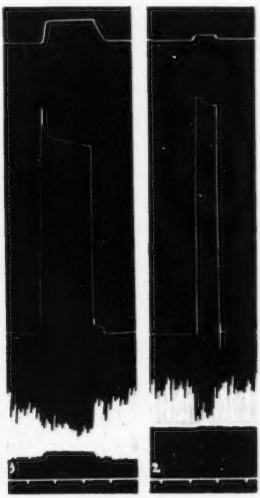

Fig. 3

Fig. 2. In 1 almost complete inhibition of knee-jerks during distention of urinary bladder (monkey). 2 demonstrates complete inhibition of these reflexes during distention of a loop of the ileum. In 3 the same phenomenon upon distention of the gallbladder. *Cm* = scale in 1 gives reduction of reproduction. *V* = record of volume of water with which viscus was distended; *P* = record of pressure (in millimeters of mercury) of distention. Time-line records intervals of 30 seconds.

Fig. 3. Inhibition of knee-jerks in spinal monkey (Thorac. VII, fifth day after transection). In 1 distention of urinary bladder, in 2, distention of a loop of ileum. Same recording as in figure 2.

What is the significance of this phenomenon? Excessive distention of the bladder or the gut elicits in the conscious human being a typical

sensation of dull pain. Everybody has experienced this sensation and knows that during such an attack of abdominal pain one bends forward and draws up his legs, i.e., a massive flexion reflex of the legs occurs. It is not unplausible to assume that a similar mechanism, though probably less evident, is operating in the anesthetized animal. As a matter of fact it was found that a weak flexion reflex sometimes ensues in the narcotized monkey upon distention of a loop of gut and that flexion reflexes rhythmically elicited by electrical stimulation of a homolateral afferent nerve are sometimes found to be slightly enhanced during distention of a loop of the ileum. Since it is well known that during a flexion reflex the extensors are inhibited, we may look upon the inhibition described in this paper as a concomitant phenomenon in a flexion reflex, elicited during the distention of the viscus. It is interesting that this inhibitory component is more pronounced, at least in the anesthetized animal, than the fundamental nervous mechanism to which it is correlated.

SUMMARY

1. Distention of the urinary bladder, the gallbladder, or a loop of the ileum of the narcotized monkey and cat results in a marked inhibition of the knee-jerk.

2. This inhibition is more pronounced in the monkey than in the cat. The position of the animal is also of influence; the phenomenon is more marked in the prone position than when the animal is in the supine position.

3. This inhibition is interpreted as an epiphenomenon, inhibition of extensor mechanisms, in a flexion reflex, elicited during the distention of the abdominal organs mentioned above.

THE BASAL INSULIN REQUIREMENT OF DEPANCREATIZED DOGS

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The basal insulin requirement of depancreatized dogs is an important subject in which little work has been completed up to the present time. Houssay, Lewis and Foglia (1) gave insulin[1] by constant intravenous injection to dogs immediately after pancreatectomy and found a rate of 0.01 unit per kilo per hour kept the blood sugar level normal. They did not make observations beyond 36 hours after operation. The work to be reported here deals with the basal insulin requirement under standard conditions and over an extended period of time.

PROCEDURE. After pancreatectomy the animals were carried on insulin for one month to allow for complete recovery. Thereafter they were maintained at a constant weight on insulin and a measured diet of raw pancreas, meat and sugar with adequate vitamins and a small amount of sodium chloride. Infection has a profound influence on the action of insulin and great care was taken to eliminate this source of variability. The animals were not used for the test if they showed any signs of infection such as bloody stools, diarrhea, nasal discharge, lack of appetite, listlessness or loss of weight. A given dog was not used more than once a week.

For the test the dog received his last food and insulin 24 to 36 hours before the start of the determination. Some insulin was given intravenously the previous evening. On the morning of the experiment two or more units of insulin were given intravenously. The purpose of this was to bring the blood sugar level down to normal; the amount needed was established by previous trial, and once determined was quite constant for a given animal. Blood sugar determinations were made every 30 minutes by the Folin-Wu micro method. After the blood sugar had reached a

[1] We wish to express our appreciation for the generous gifts of insulin from the Eli Lilly Company, and for nembutal from the Abbott Laboratories.

This work was aided by grants for technical assistance from the National Youth Administration.

normal or subnormal level and was rising again, a dilute solution of insulin in normal saline was injected intravenously either continuously or hourly, in amounts just sufficient to maintain a constant blood sugar level. After six hours of this continuous or hourly injecting the determination proper was started since by that time the animal could be considered as in a "steady state."

Figure 1 illustrates the typical manner by which the basal rate was obtained in a given dog. The broken line represents a preliminary "scouting" experiment. Two units of insulin had been given intravenously at 8:30 a.m. at which time the blood sugar was 400. The charted points

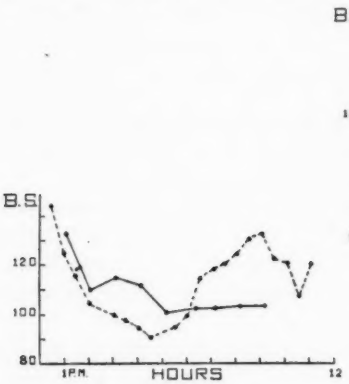

Fig. 1

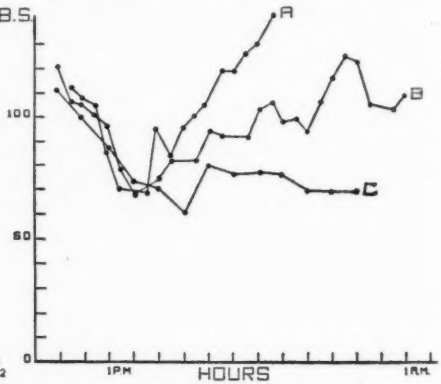

Fig. 2

Fig. 1. Comparison of intermittent and constant injections of insulin. Interrupted line gives results with intermittent injections with an average of approximately 0.03 unit per hour from 2 p.m. to 10 p.m. The continuous line gives results with constant injection of insulin at the rate of 0.031 unit per hour from 2 p.m. to 9 p.m.

Fig. 2. Curves showing blood sugar values resulting from injections at three different rates; *A* at a rate of 0.04 unit per hour, *B* at 0.06 unit per hour and *C* at an average rate of 0.072 unit per hour.

represent blood sugar values. The blood sugar had fallen to normal at 10:30 o'clock and had risen again to 145 by noon. Hourly insulin was then started at the rate of 0.06 unit which brought the level to 105. The rate was changed to 0.037 unit per hour and maintained until 4:30. This rate caused a slight fall. From 4:30 until 7:00 a rate of 0.02 unit per hour led to a definite rise and the trend was reversed by the injection of 0.035 unit per hour between 7 and 10 at which time the injection was stopped. If we divide the interval of 8 hours (from 2 p.m. to 10 p.m.) by the total insulin injected during this time we obtain an average of slightly over 0.03 unit per hour. With this information available it was easy to carry out

the next determination. This is represented by the continuous line. Four units were given at 7 a.m. when the blood sugar was 454. Constant intravenous injection was started at 11:30 at a rate of 0.1 unit per hour until 2 p.m. at which time the blood sugar was 110. From then until 9 p.m. the blood sugar level was kept within a very narrow range by the continued injection at an average hourly rate of 0.031 unit per hour. This was taken as the proper rate for the animal for this day.

The foregoing results also indicate that there is very little difference between the requirements as determined by intermittent hourly injection and that determined by constant intravenous drip. This has been our experience throughout. If anything the constant injection method gives slightly lower figures than the intermittent hourly method.

A fact that became apparent early in our work was that the insulin requirement rate of any individual dog is quite definite. If a lower rate than this be administered the blood sugar level will rise, whereas a higher than adequate rate will cause the blood sugar to pass to subnormal values. This is illustrated in figure 2 giving the course of the blood sugar in three experiments on the same dog at different insulin rates, curve *A* shows a rather rapid rise with a dosage of 0.04 unit per hour, curve *B* a more gradual rise with a dosage of 0.06 unit per hour, and curve *C* shows a very slight downward trend with an intermittent dosage averaging 0.072 unit per hour. It is obvious that the actual insulin rate for this dog must be close to 0.07 unit per hour.

Table 1 summarizes the basal insulin requirement of depancreatized dogs of various weights, expressed as units per kilo per hour and determined by the method described above. The constancy of the rate for a given animal is demonstrated by dog T which over a period of ten months varies within quite narrow limits. There is greater variation from animal to animal, on a weight basis, the lowest rate found being 0.0051 and the highest 0.035 unit per kilo per hour.

Discussion. The average requirement for all dogs is 0.0169 unit per kilo per hour. This value for the basal insulin requirement if applied on a weight basis would give a figure of 1.014 unit per hour for a 60 kgm. man. We may infer that the islet tissue is secreting insulin at some such rate in the normal fasting individual and that the fasting, completely diabetic would need the administration of such an amount to maintain his blood sugar at a normal level.

The measurement of basal insulin requirement is of practical value in experimental work in nutrition on depancreatized dogs. Such feeding experiments are ordinarily carried out on a 24-hour basis, during which time there is an absorptive, and a post-absorptive (approaching basal) period. During the latter period insulin is needed to prevent the excessive new formation of glucose. It is this requirement which is studied in the work

TABLE 1

Basal insulin requirement of depancreatized dogs

Dog T. 9.5 kgm.	Depancreatized 3/7/35. Female
6/25/1935	0.0107 unit per kilogram per hour
10/30/1935	0.0088 unit per kilogram per hour
11/ 6/1935	0.0104 unit per kilogram per hour
11/13/1935	0.0084 unit per kilogram per hour
11/27/1935	0.0081 unit per kilogram per hour
12/ 4/1935	0.0078 unit per kilogram per hour
1/ 4/1936	0.0087 unit per kilogram per hour
4/ 8/1936	0.0073 unit per kilogram per hour
Dog S. 15 kgm.	Depancreatized 9/27/35. Female
11/ 9/1935	0.0126 unit per kilogram per hour
11/16/1935	0.013 unit per kilogram per hour
11/23/1935	0.014 unit per kilogram per hour
11/29/1935	0.014 unit per kilogram per hour
12/ 6/1935	0.014 unit per kilogram per hour
Dog Bl. 15 kgm.	Depancreatized 10/24/35. Female
11/15/1935	0.0133 unit per kilogram per hour
Dog B. 9.5 kgm.	Depancreatized 12/5/35. Male
1/16/1936	0.0081 unit per kilogram per hour
Dog P. 6 kgm.	Depancreatized 2/26/36. Female
3/16/1936	0.0053 unit per kilogram per hour
4/ 8/1936	0.0051 unit per kilogram per hour
Dog Ty. 4.6 kgm.	Depancreatized 5/17/36. Female
10/ 3/1936	0.03 unit per kilogram per hour
1/ 6/1937	0.03 unit per kilogram per hour
Dog N. 6.9 kgm.	Depancreatized 5/29/36. Female
6/17/1936	0.018 unit per kilogram per hour
6/24/1936	0.018 unit per kilogram per hour
Dog W. 10 kgm.	Depancreatized 7/7/36. Male
9/ 3/1936	0.022 unit per kilogram per hour
Dog Bf. 10 kgm.	Depancreatized 7/29/36. Male
10/24/1936	0.032 unit per kilogram per hour
10/31/1936	0.031 unit per kilogram per hour
Dog H. 8.6 kgm.	Depancreatized 9/22/36. Male
10/17/1936	0.032 unit per kilogram per hour
10/10/1936	0.028 unit per kilogram per hour
Dog Hy. 14.5 kgm.	Depancreatized 10/15/36. Female
11/21/1936	0.030 unit per kilogram per hour
12/31/1936	0.035 unit per kilogram per hour
Dog K. 9 kgm.	Depancreatized 12/16/36. Male
1/ 9/1937	0.0155 unit per kilogram per hour
1/16/1937	0.0177 unit per kilogram per hour

The author wishes to acknowledge his appreciation to Dr. Jay Palmer and Mr. Hyman Bergman of the Department of Physiology who have generously supplied him with some of the figures in this table.

reported in this paper. In feeding experiments therefore not only should the relatively large insulin requirements of the absorptive period be taken care of, but also the small but constant need of post-absorptive state.

SUMMARY

A method for the determination of the constant insulin requirement of depancreatized dogs under basal conditions is described. This requirement varies between 0.0051 and 0.035 unit per kilo per hour in different animals.

If insulin be given at a rate higher than this the blood sugar will steadily fall; if given at a rate lower than this the blood sugar will steadily rise.

The requirement for any particular dog is remarkably constant over periods of time extending up to ten months.

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FURTHER EVIDENCE OF CEREBELLAR INFLUENCE IN THE HABITUATION OF AFTER-NYSTAGMUS IN PIGEONS

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The observation that under appropriate conditions repetitive elicitation of a given response may yield an "extinction" or habituation effect has been repeatedly substantiated by well controlled experiments. But the origin of this habituation effect is the subject of much controversy in the literature. Basing their position upon the studies of Hecht (1926–1927), Adrian (1932), Hoagland (1935) and others, many recent writers have included the phenomena of habituation under the term *sensory adaptation*. Others, for example Camis (1930), impressed doubtless by the gross similarities between habituation and *fatigue*, have postulated that the former is due to fatigue either of the receptor, of the effectors involved or of both. An important point of agreement is to be noted between the foregoing positions, viz., that habituation is chiefly a *peripheral* affair. Opposed to this general view is that held by a growing number of investigators: That the phenomena of habituation are principally *central* in their origin. Until quite recently, evidence in support of the central theory was largely inferential. Thus, Maxwell, Burke and Reston (1922) discarded both the notion of fatigue and of pathology when they discovered that rabbits habituated to rotation at a certain rate gave a normal nystagmic response to subsequent aural irrigation and to a "faster rate of turning." Dunlap (1925), on the other hand, found that rabbits habituated to caloric stimulation of the labyrinths exhibited some evidence of habituation to rotary stimulation as well, but observed that the two effects were differentially retained. Histological analysis by Gould (1926) of the labyrinths of healthy rats habituated in experiments on the effects of prolonged rotation, yielded only negative evidence of pathology or developmental anomaly. King (1926), whose experiment was predicated upon neurological premises since shown to be of dubious validity, was unable to demonstrate any effect of decerebration upon habituation of after-nystagmus in squabs. That birds rotated while completely anesthetized

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failed to show any of the habituation effect evidenced by controls has been reported by Fearing and Mowrer (1934).

Direct evidence of a central component in the habituation of after-nystagmus was reported for the first time by one of us (Halstead, 1935). This investigator found that pigeons with chronic cerebellar lesions (thermo-cautery) failed to habituate as readily as normal pigeons although some habituation was noted. Furthermore, pigeons which received a cerebellar lesion after habituation had previously been established, showed a marked loss of the effect following the operation. Evidence against a fatigue interpretation of habituation is contained in the additional observation that residual signs of habituation in some animals could be detected as long as three months after the end of the rotational period; however, a few animals had exhibited a reconstitution of normal nystagmus duration after one month. This apparent reversibility of the behavioral modification associated with habituation is in line with Gould's negative findings above concerning pathology of the receptor. The importance of repeating Halstead's observations has led to the present study which, in general, confirms and extends his findings.

METHOD. Experiment I: Ten adult pigeons (*Columba domestica*), all of which had one month prior to rotation received a cerebellar lesion (thermo-cautery), and ten normal controls were habituated by repeated elicitation of after-nystagmus according to the technique described by Halstead (1935), with the following modifications:

1, timing of the nystagmus was done directly by two observers by means of split-second stop watches; 2, with the exception of the first trial, the after-nystagmus time for each bird was recorded for every third elicitation; 3, instead of distributing the rotational intervals over a period of 10 to 14 days, each group of birds received all of the rotation in two experimental sessions, these sessions being separated by a rest interval of two weeks. A report comparing the two methods of presentation of the rotational experience (i.e., "massed" vs. "distributed") is in preparation. The cerebellar lesions ranged from 9 per cent to 23 per cent (average 16.7 per cent) of the total mass of the cerebellum. Such animals are essentially normal in their general behavior (Halstead, 1935). The rotator employed accommodated six animals simultaneously. During rotation, half of any given group were operated animals with their identity unknown to at least one of the observers.

Experiment II: It had been found in experiment I that the most reliable portion of the habituation curve covered 58 elicitations. Accordingly, 20 pigeons were habituated by 58 elicitations of after-nystagmus. The animals were next divided into two groups of ten animals each, equated on the basis of their nystagmus times on the last ten elicitations. One group (indicated as *cerebellar* in fig. 2) was given the standard cerebellar operation with lesions that ranged from 15 per cent to 41 per cent and averaged 29 per cent. Following the operation, this group of animals (now nine) along with controls remained in their cages for a period of two

weeks without any additional rotational experience. At the end of this period, both cerebellar and control animals were re-habituated by a second set of 58 elicitations of after-nystagmus. At the end of the last rotation period, all operated and some control animals were sacrificed and prepared for histological analysis of the lesions.

RESULTS. Figure 1 shows habituation and retention curves for the animals in the first experiment. It may be noted that the presence of a cerebellar lesion appears to influence the rate at which habituation is established. But how much of this effect is due to the fact that the two groups were different as to duration of after-nystagmus at the outset? The first point, and every fourth point thereafter, on the habituation curves represents the average of four nystagmus times for each of ten

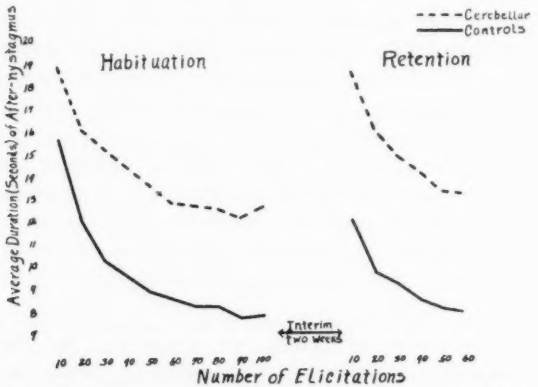

Fig. 1. Showing the influence of cerebellar lesion upon the establishment of habituation of after-nystagmus in pigeons and upon the retention of the habituation effect. (N = 10 cerebellar; 10 controls.)

birds, i.e., 40 readings (the intermediate points 30 readings). While this procedure makes for high reliability of each point on the curve, in the presence of a differential rate of change between two groups it would produce an artifactual difference in the first point of the two curves. That this explanation adequately accounts for the difference apparent at the outset of rotation is shown by an analysis of the average first reading obtained for the two groups. In table 1, M_O represents this average. The critical ratio of 0.83 suggests that the difference of 1.4 second between the two averages is not a true difference and that the two groups were actually alike with respect to duration of after-nystagmus at the beginning of rotation. This fact (in line with Halstead's previous findings) is clearly of considerable significance in interpreting the relationship of the cerebellum to the habituation process. It is also evident from figure 1 that the habit-

uation effect is retained differentially by the two groups. Whereas the cerebellar group has lost all evidence of habituation by the end of two weeks without rotation, the control group still shows marked habituation

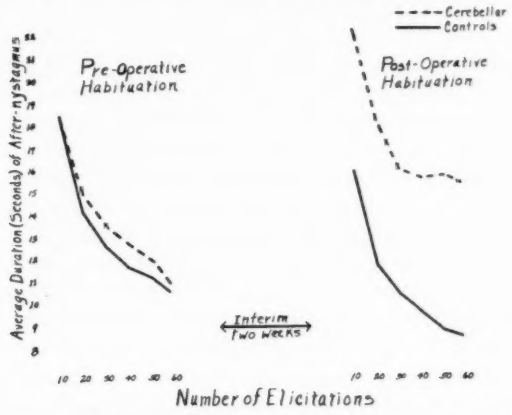

Fig. 2. Showing the influence of cerebellar lesion in pigeons upon the retention of habituation of after-nystagmus effect established prior to the operation. (N = 9 cerebellar; 10 controls.)

TABLE 1

Showing mean after-nystagmus times (seconds) for end-points on the habituation and retention curves in figures 1 and 2, with critical ratios (difference/σ difference) for same

	EXPERIMENT I					EXPERIMENT II				
	M_0 (first trial)	M_1 (habituation)	M_2 (habituation)	M_3 (retention)	M_4 (retention)	M_0* (first trial)	M_1* (habituation)	M_2* (habituation)	M_3 (retention)	M_4 (retention)
Cerebellar	20.5	18.9	12.6	18.6	13.1	21.4	18.4	10.7	22.2	15.3
Controls	19.1	15.7	7.7	12.0	7.9	23.4	18.4	10.5	16.0	8.5
Difference	1.4	3.2	4.9	6.6	5.2	2.0	0	0.2	6.2	6.8
$\frac{\text{Difference}}{\sigma \text{ difference}}$	0.83	3.70	6.19	6.80	6.00	1.13		*	6.9	9.8

* Pre-operative.

at the end of this period. It is of interest that the rate at which their previous degree of habituation is reëstablished is now the same for both groups.

In figure 2 the effect of the cerebellar lesion is manifested by 1, a com-

plete loss of habituation effect; 2, the slower rate at which a previous level of habituation is reëstablished. Expressed in terms of table 1, experiment II, the ratios of the means of the two groups reveal that whereas prior to operation the two groups were alike as to rate and degree of habituation, the cerebellar operation rendered that group quite different from the control group as to degree of habituation retained. The difference between the two groups was relatively increased by further rotation.

Discussion. Control experiments have shown that the above phenomena are independent of such factors as environmental temperature, body temperature, anesthesia, the operation, time for recovery following operation and lesions outside the cerebellum including damage to the receptor. One of us (Yacorzynski) has studied the effects of deep cerebral lesions upon the phenomena observed here. These data (unpublished) strongly suggest a diencephalic component in the habituation process which is distinguishable quantitatively from the cerebellar component reported previously by Halstead and affirmed in the present study.

As to the possible mechanisms whereby the cerebellum manifests its influence in the habituation of nystagmus, the theory of inhibition suggested by Hoagland (1935, P-102) demands careful consideration.

Janjowskaja's investigations, cited by Orbeli (1935), on the changes in blood chemistry in chronic cerebellar animals are also of considerable interest in this connection. He found a significant increase in K content of the blood, along with a corresponding shift in the normal K/Ca ratio.

Since on the behavior side we are dealing with phenomena that are indistinguishable from those generally considered as *learning* (Halstead, 1935), the importance of further investigation of the possible relationships to a chemical mechanism via the cerebellum is at once apparent.

SUMMARY

Evidence has been presented in the foregoing which confirms and extends the previous work of Halstead (1935). This evidence indicates that:

1. Pigeons with cerebellar lesions do not become habituated to repeated elicitation of after-nystagmus as readily as controls; they do not retain the habituation effect as long as controls; they re-habituate to a previous level as readily as controls.

2. Pigeons given a cerebellar lesion after habituation has previously been established show marked loss of habituation effect in comparison with controls and do not re-habituate to a previous level as readily as controls.

3. Habituation of after-nystagmus is a process involving a modification within the central nervous system, in this instance in the cerebellum.

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THE LATE EFFECTS OF BILATERAL RESECTION OF THE SPLANCHNIC NERVES ON THE HUMAN GASTRIC MOTOR MECHANISM

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In previous publications Barron, Curtis and Haverfield (1935) (1936) reported that subsequent to unilateral and bilateral resection of the splanchnic nerves in man, there ensued intense, persistent gastric motility, with a marked increase in the amplitude of the contractions. This was attributed to the unopposed action of the vagus nerves. These observations were made by the balloon and kymograph method.

Since this report the gastric motility has been reinvestigated approximately seven months postoperative. The early effects of bilateral resection of the splanchnic nerves on the human gastric motor mechanism have also been studied on another patient. In addition to the kymograph tracings, observations were made on the emptying time of the stomach. The purpose of this paper is to report these findings.

METHOD. The technic for investigating gastric motility, the indications for splanchnic resection, and the criteria for evaluating gastric motility have been presented in detail elsewhere (Barron, Curtis, and Haverfield, 1936).

The test meal utilized for determining the total emptying time of the stomach consisted of three ounces of cream of wheat, two ounces of barium sulphate and sufficient water to form a paste. Frequent fluoroscopic observations were made until barium was no longer evident in the stomach. Five observations were made during the control period; five, subsequent to unilateral, and six following bilateral resection of the splanchnic nerves.

RESULTS. Nine kymographic observations were made seven months after bilateral splanchnic resection. These revealed a marked decrease in the duration of periods of gastric quiescence, which at times was even absent during an entire period of observation. In many instances a very short period of quiescence occurred following which increased motility appeared. The longest period of gastric quiescence was 73 minutes. The specific details are tabulated in table 1.

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TABLE 1

Analysis of experiments on gastric motility seven months after bilateral resection of the splanchnic nerves

DATE OF EXPERIMENT	DURATION OF EXPERIMENT	PERIODS OF QUIESCENCE		PERIODS OF ACTIVITY		CONTRACTIONS					REMARKS
		Number	Duration	Number	Duration	Type	Number	Duration	Interval between	Amplitude	
9-26-35	3 hr., 30 m.*			1	34 m.	I	Inestimable	Inestimable	Inestimable	max.* 3 cm min*. 1 cm	Period terminated in tetany
		1	25 m.	1	151 m.	II	185	max. 35 s.* min. 5 s.	max. 40 s. min. 5 s.	max. 5.5 cm. min 3 cm.	
9-27-35	4 hr. 10 m.			1	46 m.	II and III	Inestimable	Inestimable	Inestimable	max. 4 cm. min. 2 cm.	
		1	12 m.	1	192 m.	II and III	Inestimable	Inestimable	Inestimable	max 4.5 cm. min 2 cm.	
9-29-35	5 hr.			1	93 m.	II and III	Inestimable	Inestimable	Inestimable	max. 4 cm. min 1.5 cm.	
		1	24 m.	1	109 m.	I and II	Inestimable	Inestimable	Inestimable	max. 6 cm. min 2 cm.	
		1	15 m.	1	59 m.	II	41	max. 55 s. min 20 s.	max. 3 m. min 30 m.	max. 5.5 cm. min. 2 cm.	
9-30-35	3 hr., 30 m.			1	210 m.	II	211	max. 1 m., 40 s. min. 5 s.	max. 1 m. min. 5 s.	max. 10 cm. min. 2.5 cm.	Intense motility
10- 1-35	5 hr.			1	121 m.	II	104	max. 1 m., 10 s. min. 5 s.	max. 2 m., min. 5 s.	max. 14 cm. min. 3 cm.	Intense motility
		1	35 m.	1	100 m.	II	81	max. 1 m., 5 s. min. 10 s.	max. 3 m. min 5 s.	max. 12 cm min 3 cm.	Intense motility
		1	35 m.	1	9 m.	I	Inestimable	Inestimable	Inestimable	max. 5 cm min. 2 cm.	
10- 2-35	3 hr., 25 m.			1	81 m	II	77	max. 1 m., 20 s. min. 15 s.	max. 2 m., 30 s. min. 5 s.	max. 9 cm. min. 4 cm.	
		1	16 m.	1	46 m.	I	Inestimable	Inestimable	Inestimable	Low grade	
				1	62 m.	II	48	max. 1 m., 10 s. min. 15 s.	max. 3 m. min. 5 s.	max. 11.5 cm. min. 3 cm.	Intense motility

* m.—minutes, max.—maximum, s.—seconds, min.—minimum.

TABLE 1—*Concluded*

DATE OF EXPERIMENT	DURATION OF EXPERIMENT	PERIODS OF QUIESCENCE		PERIODS OF ACTIVITY		CONTRACTIONS					REMARKS
		Number	Duration	Number	Duration	Type	Number	Duration	Interval between	Amplitude	
10- 3-35	5 hr.			1	64 m.	II	56	max. 2 m. min. 5 s.	max. 1 m., 30 s. min. 5 s.	max. 7.5 cm. min. 2 cm.	Period terminated in tetany
		1	73 m.	1	163 m.	II	141	max. 1 m., 5 s. min. 10 s.	max. 2 m. min. 5 s.	max. 8 cm. min. 2.5 cm.	Intense motility
10- 4-35	4 hr.	1	26 m.	1	113 m.	I and II	Inestimable	Inestimable	Inestimable	max. 7 cm. min. 2 cm.	Intense motility
		1	17 m.	1	84 m.	I and II	Inestimable	Inestimable	Inestimable	max. 9 cm. min. 2.5 cm.	Intense motility
10- 5-35	5 hr., 5 m.			1	79 m.	II	76	max. 2 m. min. 5 s.	max. 1 m., 30 s. min. 5 s.	max. 5 5 cm. min. 2 cm.	Period terminated in tetany
		1	59 m.	1	142 m.	II	110	max. 1 m., 35 s. min. 5 s.	max. 2 m., 30 s. min. 5 s.	max. 7 cm. min. 3 cm.	Period terminated in tetany
		1	25 m.								

There was a marked increase in the duration of gastric motility which occasionally persisted throughout an entire observation period. Associated with this there was an increase in the number of contractions. Although frequently these occurred with great rapidity, the number of contractions, when possible, was estimated and compared with the control data which have already been published (fig. 1, Barron, Curtis and Haverfield, 1936).

The amplitude of the contractions was markedly increased. Contractions frequently measured from 7 to 14 cm. (fig. 2). Many periods of motility terminated in severe gastric tetany.

These results show that intense gastric motility still persisted. In this respect there was but little variation from the immediate postoperative results. There was also no change in the duration of and the interval between contractions. It would seem from these observations that the effects of unilateral and bilateral resection of the splanchnic nerves persisted for at least seven months.

Kymographic observations on the effect of splanchnic resection on the gastric motility of another patient confirmed in their entirety the results previously reported (Barron, Curtis and Haverfield, 1936) and therefore the specific data are intentionally omitted.

Studies were also made on the influence of bilateral splanchnic resection

on the total emptying time of the stomach. Five observations were made during the control period. The results (table 2) were fairly uniform. The normal emptying time varied from four hours and fifteen minutes to five hours and twenty-five minutes, and averaged four hours and forty-five minutes.

Although the results following unilateral splanchnic resection showed considerable variability, the average emptying time revealed practically

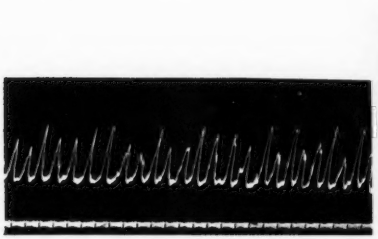

Fig. 1

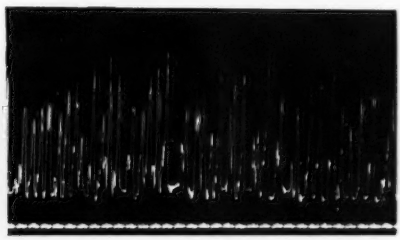

Fig. 2

Fig. 1. Tracing of gastric motility during the control period.

Fig. 2. Tracing made seven months after bilateral resection of the splanchnic nerves showing intense gastric motility.

TABLE 2

The influence of bilateral resection of the splanchnic nerves on the emptying time of the human stomach

CONTROL STUDIES	UNILATERAL SPLANCHNIC RESECTION	BILATERAL SPLANCHNIC RESECTION
4 hr., 20 min.	6 hr.	4 hr.
4 hr., 15 min.	4 hr., 35 min.	3 hr., 5 min.
5 hr., 25 min.	5 hr., 15 min.	4 hr., 15 min.
5 hr.	4 hr.	3 hr., 30 min.
4 hr., 45 min.	3 hr., 35 min.	2 hr., 45 min.
		3 hr., 25 min.
Average...4 hr., 45 min.	4 hr., 41 min.	3 hr., 30 min.

no change when compared with the results of the control period (table 2). The total emptying time ranged from three hours and thirty-five minutes to six hours and averaged four hours and forty-one minutes, an average difference of only four minutes.

Six observations on the emptying time of the stomach made subsequent to bilateral splanchnic resection revealed a variation which ranged from two hours and forty-five minutes to four hours and fifteen minutes. However, the average emptying time was three hours and thirty minutes, a difference of one hour and fifteen minutes (table 2).

Discussion. The effect of bilateral resection of the splanchnic nerves on gastric motility has been studied extensively on experimental animals by Carlson (1916), McCrea (1925) and McSwiney (1931). Our results in man are comparable to those already reported. We observed an increase in the duration of periods of motility and in the amplitude of the gastric contractions. These results were confirmed in a second patient. This increased gastric activity persisted for at least seven months subsequent to bilateral splanchnic resection. However, Cerqua (1935) reported that the stomach of dogs appeared less active after splanchnic section.

Meek and Herrin (1934) have shown that the consistency of the test meal influenced the rate of gastric emptying. We therefore selected a semisolid meal since in our experience this eliminated many discrepancies already reported (McCrea, 1925).

Our results revealed that subsequent to unilateral resection of the splanchnic nerves, there was no change in the average rate of gastric emptying. Following bilateral resection there was a decrease in the emptying time. These results coincide with those of McSwiney (1931).

These observations would seem to indicate that the decrease in the emptying time of the human stomach is due at least in part to the increased gastric activity which ensued subsequent to bilateral resection of the splanchnic nerves.

CONCLUSIONS

Observations on the gastric motor mechanism in two patients before and after bilateral splanchnic resection revealed a marked increase in the duration of periods of gastric motility and a definite increase in the number and amplitude of the contractions. This persisted for at least seven months.

The total emptying time of the stomach was not influenced by unilateral splanchnic resection. Subsequent to bilateral splanchnic resection the average emptying time was three hours and thirty minutes, an average difference of one hour and fifteen minutes.

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INFLUENCE OF ADRENALECTOMY ON LIVER FAT AS VARIED BY DIET AND OTHER FACTORS

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Removal of the adrenal glands has been found (1) to reduce or abolish ketosis due to various causes. In considering ketosis as a disturbance of carbohydrate metabolism Geelmuyden (2) described the chain of events as involving fat migration, a fatty liver and then ketosis. Deuel et al. (3) have also suggested a relationship between the amount of fat in the liver and the height of the fasting ketosis, and we found that adrenalectomy not only abolished the ketosis due to ketogenic anterior pituitary extracts (1), but also prevented the deposition of fat in the liver (4) which results when such extracts are administered to normal animals (5). These observations and the reports that adrenalectomy prevents the fatty liver which usually accompanies total pancreatectomy (6) as well as in phosphorus poisoning (7) led us to examine the influence of adrenalectomy upon the transport of liver fat as varied by the diet.

The adrenals were removed from rats as usual (8) under ether anesthesia. Controls were given ether but sham operations not performed. The rats were etherized and then bled from the large abdominal vessels to drain the excess blood from the liver. In some experiments (expts. 1–5) the livers were dried, powdered and extracted with petroleum ether to obtain their fat content. In experiments 6 and 7 the fatty acids and non-saponifiable lipids, as a measure of the fat content, were determined by alkaline digestion of the livers with resulting saponification of the fat, and after acidification, extraction with petroleum ether for drying and weighing. Other details of the various experiments are noted in table 1.

In simple fasting adrenalectomy reduces (expt. 1) the amount of fat which is contained in the liver, an observation which has been made before (4). An alkalosis is known to produce a ketosis (9, 10). This ketosis practically disappears after adrenalectomy (expt. 2). Since it produces a ketosis it is not surprising that an alkalosis due to the administration of sodium bicarbonate increases the amount of fat in the liver of the fasting rat (expt. 2, group C) above the level which would otherwise be found (expt. 1, group C). After adrenalectomy this alkalosis fails to

TABLE 1

EXPERIMENT	GROUP	NUMBER OF RATS	SEX	BODY WEIGHT	BODY SURFACE	LIVER WEIGHT		LIVER FAT		DAYS OF FASTING	DAYS AFTER ADRENALECTOMY	KETONE BODY EXCRETION PER 100 SQ. CM. BODY SURFACE PER DAY
						Observed	Per 100 sq. cm. body surface	Per cent	Per 100 sq. cm. body surface			
				grams	*sq. cm.*	*grams*	*grams*		*mgm.*			*mgm.*
1. Fasted two days, operated upon and fasted for two days more	C	5	♀	162	337	4.58	1.36	2.64	36	4		
	Ad.	5	♀	161	335	4.92	1.47	1.80	26	4	2	
2. Fasted two days, operated, then given orally 2 cc. of 7.5% $NaHCO_3$ twice daily for 2 days	C	5	♀	148	316	4.21	1.33	5.96	79	4		8
	Ad.	5	♀	148	316	4.44	1.40	0.77	11	4	2	0.1
3. Fasted 2 days, operated, then for 2 days more each rat given 0.5 mgm. epinephrine 3 × daily subcutaneously	C	5	♀	160	334	4.38	1.31	4.79	63	4		
	Ad.	5	♀	162	337	4.06	1.21	0.96	12	4	2	
4. Fasted 1 day, operated, then given 2 cc. per 100 sq.cm. body surface of 40% cream containing 3% $NaHCO_3$ twice daily for 3 days	C	6	♀	202	391	5.95	1.52	4.00	61	0		
	Ad.	7	♀	186	370	5.87	1.59	1.31	21	0	3	
5. Operated without previous fasting and given 3 cc. per rat per day of a mixture of 50% butter and 50% cottonseed oil daily for 2 days	1C	6	♂	186	370	5.30	1.43	5.28	75	0		27
	2C	6	♀	162	337	5.01	1.49	7.50	112	0	2	55
	1Ad.	6	♂	190	375	5.68	1.51	3.42	51	0		0.5
	2Ad.	6	♀	164	339	5.61	1.65	3.36	55	0	2	0.1
6. Received no other food but 40% cream containing 3% $NaHCO_3$ *ad lib* for 8 days, then operated upon and fasting begun	1C	5	♀	138	304	5.58	1.83	15.05	276	0		
	2C	3	♂	155	327	5.18	1.59	12.43	198	0		
	3C	6	♀	138	304	4.00	1.32	9.35	123	2		
	4C	3	♂	149	318	4.44	1.40	8.61	120	2		
	5C	6	♀	126	286	3.55	1.24	7.13	89	4		
	3Ad.	5	♀	134	298	3.97	1.33	4.92	65	2	2	
	4Ad.	3	♂	150	321	4.50	1.40	4.61	64	2	2	
	5Ad.	6	♀	130	292	3.46	1.19	2.56	30	4	4	
7. On a low protein fatty liver producing diet for 20 days, then operated upon and fasting commenced	1C	4	♂	212	404	7.23	1.79	14.10	252	0		13
	2C	3	♂	203	392	5.55	1.41	11.86	168	2		23
	3C	3	♂	200	388	5.43	1.40	9.98	139	3		11
	4C	4	♂	200	388	4.39	1.13	8.12	92	4		
	5C	5	♀	168	345	5.98	1.73	16.40	284	0		
	6C	3	♀	163	338	4.19	1.24	10.01	124	2		5
	7C	3	♀	167	344	4.45	1.29	8.34	108	3		9
	8C	4	♀	165	341	4.16	1.22	6.19	76	4		16
	2A	3	♂	203	392	5.78	1.48	6.03	89	2	2	4
	3A	3	♂	201	390	5.01	1.28	5.31	68	3	3	5
	4A	4	♂	197	384	4.35	1.13	4.40	50	4	4	2
	6A	3	♀	165	341	4.68	1.37	6.28	86	2	2	0.2
	7A	3	♀	165	341	4.18	1.23	5.47	67	3	3	1
	8A	4	♀	155	327	4.00	1.22	4.29	52	4	4	0.1

cause the deposition of fat in the liver (expt. 2). Epinephrine administration is known to produce a ketosis. It will also result in the deposition of fat in the liver but this can be prevented by the removal of the adrenal glands (expt. 3).

A diet consisting almost entirely of fat in the form of heavy cream or liquid fat will cause a ketosis in rats. They will also acquire fatty livers. Adrenalectomy abolishes the ketosis (expt. 5) and greatly reduces the amount of fat transported to the liver (expts. 4 and 5).

Since adrenalectomy prevents the deposition of fat in the liver that would normally occur under a number of conditions it becomes of interest to determine the influence of this procedure upon fat which has previously been stored there. In normal rats a cream diet (expt. 6) or Channon's low protein high fat diet (11) produces very fatty livers (expts. 6 and 7). When these rats are then fasted this excess fat gradually leaves the liver over a period of several days. If they are adrenalectomized when fasting is begun the fat stored in the liver leaves this organ much more rapidly (expts. 6 and 7).

Experiments have been designed for the purpose of inquiring into the mechanism through which the removal of the adrenal glands interferes with the deposition of fat in the liver and hastens the exodus of fat which is already stored there. Last and Verzar suggest (7) that adrenalectomy prevents the synthesis of fat to phosphatide which they believe is necessary for its transport in the organism. At present an indirect effect exerted through the influence of the adrenals on carbohydrate metabolism seems to be a more likely possibility.

Under similar circumstances the female rat has a higher level of liver fat than the male (12). This is particularly evident on a low protein, high fat diet which produces very fatty livers (13). It can be seen in several of the experiments presented here (expt. 5, groups 1 C and 2 C; expt. 6, groups 1 C and 2 C; expt. 7, groups 1 C and 5 C). A comparison of the results in experiments 5 (groups 1 C and 2 C vs. 1 Ad and 2 Ad), and 7 (groups 2 C and 6 C vs. 2 A and 6 A) would suggest that adrenalectomy removes the sex difference in the liver fat level. This is of interest for adrenalectomy also appears to abolish the sex difference in an experimental ketosis (14).

SUMMARY

Adrenalectomy in the rat reduces the amount of fat deposited in the liver, as well as the extent of the ketosis following the administration of cream or pure fat, during an alkalosis produced by feeding sodium bicarbonate or as a result of epinephrine injections. During fasting the high concentration of fat in the liver of rats, previously receiving a cream diet or a diet low in protein but high in fat, becomes reduced more rapidly if

their adrenals have been removed than in the controls. Adrenalectomy tends to abolish the sex difference in liver fat levels.

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THE EXCRETION OF AMMONIA BY THE PERFUSED KIDNEY

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Since the demonstration by Nash and Benedict (1) that in the intact animal the ammonia content of the renal venous blood is regularly greater than that of the arterial blood, it has been commonly held that the urinary ammonia originates exclusively in the metabolic activities of the kidney. This view has been partially or completely supported by other investigations (2, 3, 4, 5, 6, 7, 8). In some experiments that we have performed bearing upon the factors influencing the chloride content of the urine from the perfused kidney in the heart-lung-kidney preparation, we have had occasion to make observations upon the urinary excretion of ammonia following increases in the ammonia content of the perfusion fluid. The observations have shown that the site of origin of urinary ammonia depends upon the blood concentration of that substance, and it seems important to record these observations in order to render more complete our knowledge of the mechanism of ammonia excretion.

Nash and Benedict found that the renal vein ammonia concentration was on the average twice as great as the arterial blood level. They did not study the effect of artificially increased arterial blood ammonia upon the situation. It would, indeed, be difficult, if not impossible, to perform such experiments on the intact dog because of the fact that ammonia is so rapidly removed from the blood by the liver that it is virtually impossible to elevate the blood ammonia significantly by intravenous injections.

We have been able to raise the level to 0.4 mgm. per cent ammonia by injection for one hour at the rate of 0.25 gram ammonium carbonate per 5 minutes in the anesthetized intact dog, but larger doses are quickly fatal and higher blood concentrations are necessary in order to clearly demonstrate direct renal excretion, which is easy to observe in the isolated heart-lung-kidney. Previous studies on ammonia excretion by the kidney in this preparation were made by Bornstein and Budelmann (9). They observed production of ammonia but otherwise their results are of no interest to the present study.

METHODS. The heart-lung-kidney was prepared according to the method of Starling and Verney (10). The renal perfusion pressure was

maintained constant in any particular experiment, its level in several experiments being set at values from 100 to 150 mm. of mercury. The blood perfusing through the kidney was maintained at constant temperature by passing through a warming coil in a thermostat held at 37.5°C. All solutions introduced into the perfusion fluid were isotonic with the blood except as indicated. The concentration of ammonia in blood and urine was determined by the method described by Nash and Benedict (1). Our results agree with theirs in regard to the usual values for ammonia in the blood of normal dogs. Urine pH was determined with a glass electrode

TABLE 1

Blood and urine NH_4 and pH following injections of isotonic solutions of salts in the heart-lung-kidney preparation

EXPERIMENT NUMBER	ARTERIAL BLOOD NH_4		URINE NH_4		URINE pH		ADDITION
	Before	After	Before	After	Before	After	
	mgm. per cent	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*			
1	0.5	8.0	4.5	17.5	8.00	7.84	0.7 gram NH_4Cl
		12.0		34.0		7.78	0.7 gram NH_4Cl
		23.5		44.0		7.73	1.7 gram NH_4Cl
2	0.5	4.7	18.0	37.0			1.2 gram $(NH_4)_2CO_3$
3	0.4	7.4	4.8	15.4	8.30	8.57	1.2 gram $(NH_4)_2CO_3$
		12.1		28.3		8.60	2.0 gram $(NH_4)_2CO_3$
4	0.2	3.6	11.0	20.4			1.0 gram NH_4 acetate
		4.0		26.2			1.0 gram NH_4 acetate
5	0.3	4.6	11.7	16.9			1.0 gram NH_4 acetate
		8.4		54.5			1.0 gram NH_4 acetate
6	0.8	3.6	11.5	57.2			1.0 gram NH_4 acetate
		9.0		71.4			1.0 gram NH_4 acetate

and electron tube voltmeter. Blood was drawn from the renal vein and from the renal arterial cannula without exposure to air and immediately subjected to analysis. Urine was collected in open tubes, exposed to air, and some ammonia loss may have occurred. Our interest was primarily in the blood ammonia values, however, and any errors in the urine concentration would be similar in the several samples, which were treated alike.

RESULTS. In table 1 we have collected the values obtained for arterial blood and urinary ammonia before and after additions of ammonium salts to the system. It is regularly observed that increasing the blood ammonia

results in augmented urinary excretion. There is no fixed relation between blood and urine ammonia in the several experiments, but in any given preparation an increase in blood ammonia invariably increased urinary excretion. Three salts were employed and the effect was the same in each case.

In figures 1 and 2 are presented the results following the injection of 100 cc. quantities of isotonic ammonium acetate into the heart-lung-kidney system. The total volume of fluid and tissue in the system is

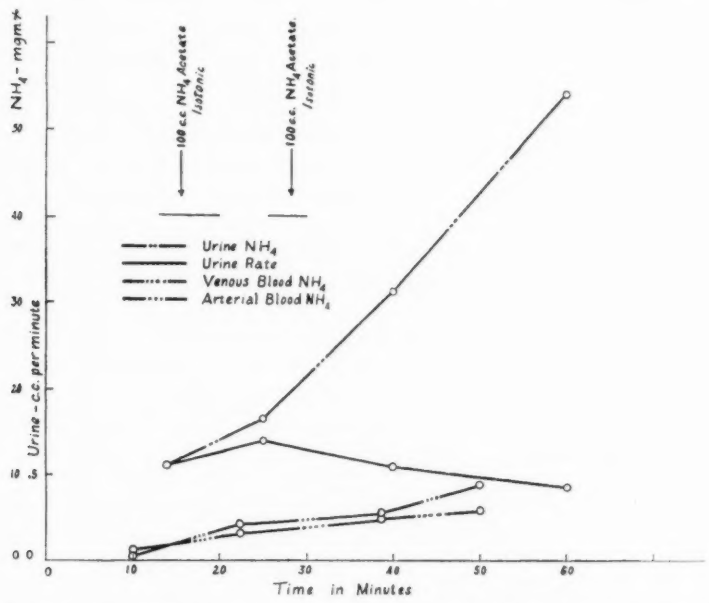

Fig. 1. Changes in urinary secretion by the heart-lung-kidney preparation after addition of ammonium acetate to the blood. Renal blood flow 110 cc. per minute, renal arterial pressure 130 mm. Hg.

approximately 2 liters. Before the first injection in figure 1 the arterial blood ammonia was 0.34. At this time the venous blood ammonia was 0.52 mgm. per cent. Following the first injection the value for arterial blood rose above that in the venous, respective figures being 4.62 and 3.94 mgm. per cent. The second addition brought a still greater rise in blood ammonia with a greater difference between the arterial and the renal venous values. Coincident with these changes there was an increase in the percentage of ammonia in the urine and an increase in the absolute quantity excreted.

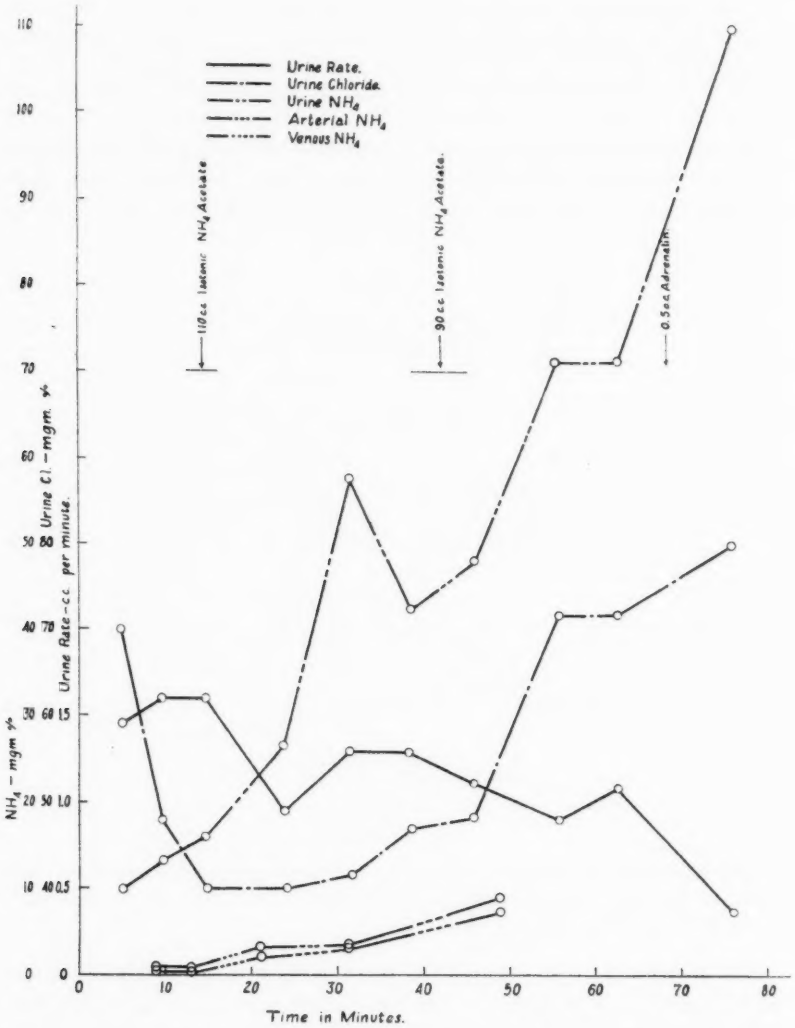

Fig. 2. Changes in urinary secretion by the heart-lung-kidney after addition of ammonium acetate to the blood. Renal blood flow 250 cc. per minute, renal arterial pressure 120 mm. Hg.

There seems to be no escape from the conclusion that in the presence of these abnormally high quantities of ammonia in the blood the kidney is capable of removing it and eliminating it in the urine.

Figure 2 presents the results of another experiment in which the results are comparable except that in this case the arterial ammonia was originally 0.80 mgm. per cent, somewhat higher than in the previous instance, and that the renal venous ammonia began at 0.60 mgm. per cent, indicating that at this level there is already an excretion of blood ammonia.

In all seven heart-lung-kidneys were used and sixteen separate additions of ammonium salts have been studied. In every instance a considerable increase in ammonia excretion occurred following ammonia injection. In the case of $(NH_4)_2CO_3$ the salt was dissolved in Ringer's solution in order that the perfusion fluid might not become hypotonic after the carbon dioxide had been excreted by the lungs, and the ammonia through the lungs and kidneys.

The urine in the heart-lung-kidney preparation is normally alkaline. Measurements with the glass electrode (table 1) have shown that the increase in urinary ammonia is not due to any consistent change in the acidity of the urine. In experiment 1 the ammonium chloride injection was followed by a less alkaline urine, in experiment 3 addition of ammonium carbonate resulted in greater alkalinity, yet in both instances the amount of ammonia excreted was greatly increased following the elevation of blood ammonia.

Discussion. It is not intended to imply from these experiments that with normal blood ammonia values the urinary ammonia is derived from that source. However, it seems quite evident that the kidney is capable of secreting and concentrating ammonia from the blood. Calculations show that after elevation of the blood ammonia level more of this substance is lost from the blood than appears in the urine as it was collected. A sample calculation may be given. For the experiment in figure 1 the renal blood flow was 110 cc. per minute. For the period at 25 minutes the arterio-venous ammonia difference was 0.68 mgm. per cent, the urine rate was 0.68 cc. per minute, and the urine ammonia 16.9 mgm. per cent. The blood loss was 0.75 mgm., and the urine excretion 0.115 mgm. per minute. Thus more ammonia is lost from the blood than is determined in the urine. As noted under Methods, however, some urine ammonia may have been lost to the air.

It seems that direct excretion does not become apparent at low levels of blood ammonia because the normal rate of ammonia production in the kidney is so great as to obscure it. Formation by the kidney is, therefore, presumably the factor of primary importance under normal circumstances, but a small proportion of the urinary ammonia, even then, may be cleared from the blood plasma. In the light of our knowledge about the mechanism of kidney excretion, it would, indeed, be strange if this were not true, because the glomerular filtrate should certainly contain ammonia at the concentration in which it is found in the plasma.

CONCLUSIONS

When the blood ammonia level in the heart-lung-kidney preparation is elevated above 0.6 mgm. per cent the renal venous blood contains less ammonia than the arterial blood. On addition of ammonium salts to the blood there is an increase in blood ammonia and a corresponding augmentation in the concentration and total excretion of ammonia in the urine, independent of the direction of the pH change. It is concluded that the kidney is able, under these circumstances, to excrete ammonia from the blood.

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SUSCEPTIBILITY OF ERYTHROCYTES TO HYPOTONIC HEMOLYSIS AS A FUNCTION OF DISCOIDAL FORM[1]

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The varied susceptibilities to hemolysis by hypotonic solutions of the erythrocytes of different species of mammals and of different pathological conditions in man have been somewhat vaguely ascribed to differences in "osmotic resistance" and in "permeability." However, the possibility that differences in the dimensions of erythrocytes might be involved has been suggested with respect to discoidal diameter by Chauffard (1) and by Vallery-Radot and Lhéritier (2) and with respect to discoidal volume by Ponder (3). Such correlations imply that the dimensions of the erythrocyte are in some way closely related to the problem, but do not necessarily depict a mechanism at work under the experimental conditions of the "resistance" test.

In 1922 Gänsslen (4) proposed a dynamic explanation of the characteristically increased susceptibility of the erythrocytes of chronic familial hemolytic jaundice to destruction by hypotonic salt solution. He pointed out that the more nearly spherical erythrocytes characteristic of this condition could increase less in volume before becoming spherical, and hence necessarily causing an extension of the surface, than could the normal, more discoidal human erythrocytes. Haden's (5) recent measurements have confirmed Gänsslen's suggestion and as a corollary have emphasized the relationship of the decreased thickness:diameter ratio of the erythrocytes of hypochromic anemia to their decreased susceptibility to hypotonic hemolysis.

Nevertheless, if differences in the form of erythrocytes are to explain entirely differences in susceptibility to hemolysis by hypotonic solutions, significant differences in strictly osmotic behavior must be shown not to exist. Moreover, for the support of Gänsslen's hypothesis it is essential to show that differences in shape can quantitatively explain differences in susceptibility to hemolysis. And, finally, further support to the geo-

[1] The expenses of this investigation were defrayed in part by the J. K. Lilly gift to the Harvard Medical School.

metrical hypothesis would be given if differences in shape, rather than in permeability, could be shown to account for differences in the time necessary for a given hypotonic solution to produce hemolysis of different types of erythrocytes (6).

EXPERIMENTS. *Percentage increases in erythrocyte volume in hypotonic plasma.* Our (7) approach to the problem was to examine the osmotic behavior of erythrocytes of different susceptibilities to hypotonic hemolysis. Determinations were made of the percentage increase in volume of samples of such erythrocytes after suspension in progressive dilutions of homologous oxalated plasma. In all, 45 observations were made on the erythrocytes of normal humans, dogs, rabbits, pigs and sheep and on the erythrocytes of patients with severe hypochromic anemia and with chronic familial hemolytic jaundice. In each instance a suitable amount

TABLE 1

Average percentage increase in equilibrium volumes of various types of erythrocytes

TYPE OF ERYTHROCYTE	NUMBER OF EXPERIMENTS	CONCENTRATION OR RELATIVE OSMOTIC PRESSURE OF PLASMA								
		1.0	0.95	0.90	0.85	0.80	0.75	0.70	0.65	0.60
Hypochromic anemia	8	0		5.8		14.3		22.8		37.1
Normal human	8	0		4.7		12.9		21.5		33.2
Dog	5	0	1.8	4.0	6.6	12.2	15.9	20.4	26.5	30.6
Hemolytic jaundice	5	0	2.6	4.6	10.7	12.9	15.9	20.1	24.3	29.8
Rabbit	5	0	2.9	4.2	9.9	13.6	17.4	23.9	H*	H
Pig	8	0	0.8	4.9	7.7	12.1	18.9		H	H
Sheep	6	0	4.1	5.2	7.8	12.9	16.9	22.3	H	H
Averages		0	2.4	4.8	8.5	12.9	17.0	21.8	25.4	32.6

* H indicates that some hemolysis was present in these concentrations after centrifuging.

of venous blood was rendered incoagulable by the addition of 0.05 cc. of a 20 per cent solution of potassium oxalate to each 5 cc. of blood. The volume percentage of the erythrocytes of each sample was then determined by means of Wintrobe (8) hematocrit tubes. On the basis of this value, homologous oxalated plasma was added to an uncentrifuged portion of the original blood in amounts sufficient to yield a sample having a cell volume of 30 per cent. Into each of a series of nine test-tubes 1 cc. of the 30 per cent erythrocyte suspension was added to such amounts of homologous plasma and distilled water that the same original volume of erythrocytes was suspended in equal amounts of concentrations of homologous plasma of 1.0, 0.95, 0.90, 0.85, 0.80, 0.75, 0.70, 0.65, and 0.60, respectively. A sample of the erythrocyte suspension in each tube was pipetted into each of two Wintrobe hematocrit tubes. All the hematocrit

tubes from one experiment were then placed in the centrifuge at the same time and rotated at 2500 r.p.m. for 30 minutes. After centrifuging, the percentage volume of erythrocytes was measured in all the hematocrit tubes showing no trace of hemolysis.

From the averages of the uncorrected duplicate hematocrit determinations were computed the respective percentage increases in the equilibrium

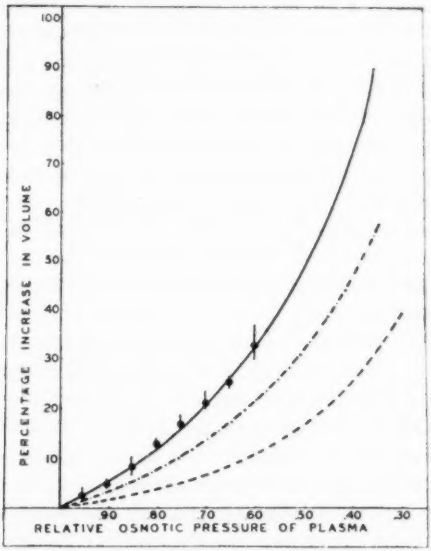

Fig. 1. Average values from 45 experiments on the percentage increase in equilibrium volume of 7 types of erythrocytes in hypotonic oxalated plasma (solid circles). The vertical line through the solid circle shows the extremes of average values obtained for any type of erythrocyte (see table 1). The upper curve (solid line) gives the best approximation to the average values and is based on the formula,

$$V_e = \left[\frac{1\ (100 - b)}{P_e} + b\right] - 100,$$

using a value of 51 for b. The middle curve is constructed from this formula, using a value of 69.4 for b. The lowest curve is described by a value of 82.9 for b. For an explanation of the theoretical significance of these curves, consult the text.

volume of the erythrocytes in each plasma concentration over their volume in undiluted plasma in the first tube. In table 1 are shown the average results of all the observations on each species and pathological condition. The final averages of these values are also shown in table 1 and are plotted as solid circles in figure 1. The vertical lines in figure 1 indicate the extremes of the average data for any type of erythrocyte.

Microscopic observations of alteration in erythrocyte form in hypotonic plasma. The data in table 1 apparently indicate the similar osmotic behavior of the various types of erythrocytes. The problem is therefore to account for the wide differences in susceptibility to hypotonic hemolysis of erythrocytes undergoing apparently similar percentage increases in volume in hypotonic plasma. Now, it is essential for the validity of Gänsslen's hypothesis that the erythrocyte in swelling behaves like a balloon with a liquid interior rather than like a sponge which swells without changing its form. On this point agreement is not complete, although Ponder and Millar's (9) (10) critical observations demonstrate a decrease in cell diameter as swelling occurs, and Haden (5) has recently found an increase in the thickness:diameter ratio of erythrocytes in dried blood films prepared from progressive dilutions of heparinized blood with distilled water. Nevertheless, it seemed desirable to observe directly the changes in form of erythrocytes of different susceptibilities to hypotonic hemolysis when suspended in various dilutions of oxalated plasma and especially when two types of erythrocytes were suspended together in the same hypotonic plasma.

A drop of a dilute suspension of any type of erythrocyte in homologous oxalated plasma was placed upon a flat slide and covered with a thin cover-glass. Immediately after being sealed with paraffin the preparation was examined under an oil immersion lens with the stage of the microscope in a *vertical* position and illuminated by a strong source of artificial light. In this way a view of different aspects of the corpuscles was obtained as they rotated or oscillated in sinking through the plasma. All types of corpuscles showed the biconcave discoidal or, rarely, the "cup" form in isotonic oxalated plasma. If, however, a suspension of any type of corpuscle was examined in hypotonic oxalated plasma of such concentration that over half of the cells were hemolyzed, many of the remaining intact cells were seen to be almost spherical but some were still somewhat biconcave in form. If serial observations were made in increasingly dilute samples of plasma, the proportion of almost or quite spherical cells increased.

That a given concentration of hypotonic plasma had a greater effect on the original form (not volume) of erythrocytes of relatively diminished resistance to osmotic hemolysis than on the form of erythrocytes possessing a greater resistance to hemolysis was best demonstrated by examination of a preparation containing two such different types of erythrocytes, for example, a mixture of sheep and human cells. When suspended in suitable human plasma of 0.5 concentration, many of the sheep cells were hemolyzed but those remaining intact were readily identifiable because of their small size when compared with the human corpuscles. Many of the sheep corpuscles were noted to be almost spherical, sometimes ex-

hibiting only a slight depression or "pip" at one point, whereas the human erythrocytes exhibited a decidedly biconcave form. If, however, human corpuscles were observed in plasma of 0.34 concentration, many of them, like those of the sheep when suspended in plasma of 0.5 concentration, were noted to have become almost spherical.

Calculation of susceptibility to hypotonic hemolysis on the basis of erythrocyte form. The qualitative behavior of the erythrocytes described above suggests that the swelling of erythrocytes as implied by Gänsslen's (4) hypothesis and by Jacobs (6) is not accompanied by increase in surface until a spherical form is attained. If this is so, the resistance to hemolysis of a given type of erythrocyte might be expressed *by the percentage difference between the original discoidal volume of the erythrocyte in isotonic solution and the volume of a sphere with surface equal to that of the erythrocyte in isotonic solution.* Accordingly, estimates were made of the geometrical characteristics of the various samples of erythrocytes whose equilibrium volumes had been studied.

From those observations the uncorrected volume percentage of erythrocytes in each oxalated venous blood sample was already known. A count of the number of erythrocytes per cubic millimeter in each sample was made, and by means of the formula,

$$\frac{\text{Cell volume percentage}}{\text{Millions of cells per cubic millimeter}} \times 10 = \text{M.C.V. (cubic micra)},$$

the uncorrected mean corpuscular volume, V_o, was calculated. From each oxalated venous blood sample, films were immediately prepared and subsequently stained with Wright's stain. From these preparations the mean cell diameter, D_o, was determined by measurement of 500 erythrocytes by the method of Price-Jones (11). The mean corpuscular thickness, T_o, was then calculated by means of the formula,

$$T_o = \frac{4\,V_o}{\pi(D_o)^2},$$

on the assumption of von Boros (12) that the erythrocyte is a right cylinder of known volume and diameter. The volume, diameter and thickness of this cylinder being known, its surface was found, and finally the volume, V_s, of a sphere with this surface. The percentage increase in volume necessary to cause the discoidal erythrocyte to assume a spherical form without change in surface would then be represented by the formula,

$$\frac{V_s - V_o}{V_o}.$$

Average data for the dimensions of each type of erythrocyte are shown in table 2.

Since it was impracticable to obtain sufficient oxalated plasma for determining the range of susceptibility to hemolysis of the erythrocytes of each sample, this information was secured by using appropriate dilutions of salt solution according to the method of Daland and Worthley (13). A mean value was taken as lying halfway between the concentrations for "definite" and "partial," as defined by these workers. In quantitative studies of hemolysis this value was shown to correspond fairly well to the steepest part of the S-shaped summation curve obtained, or to about 50 per cent hemolysis. Other observations showed that the range of susceptibility to hemolysis of normal human and sheep erythrocytes in serial dilutions of oxalated plasma corresponded sufficiently closely to that in

TABLE 2

Average venous blood values and average dimensions of various types of erythrocytes

TYPE OF ERYTHROCYTE	NUMBER OF EXPERIMENTS	VENOUS BLOOD VALUES				DIMENSIONS OF ERYTHROCYTES				
		R.B.C.	Hb	R.B.C. Volume	M.C.H.C.	V_o	D_o	T_o	S_o	V_s
		mils.	*grams*	*per cent*	*per cent*	μ^3	μ	μ	μ^2	μ^3
Hypochromic anemia	8	3.36	6.2	21.1	25.5	63.7	7.03	1.63	114.0	114.6
Normal human	5	4.85	14.5	41.1	32.4	85.7	7.18	2.11	128.3	136.6
Dog	5	6.76	15.9	48.1	33.0	66.1	6.45	2.01	106.3	103.1
Hemolytic jaundice	6	3.42	11.4	29.1	36.1	84.4	6.88	2.28	122.9	128.0
Rabbit	5	3.97	9.4	30.7	30.7	70.9	6.22	2.34	106.7	103.4
Pig	4	8.23	14.6	45.3	32.3	51.9	5.47	2.21	85.3	74.2
Sheep	4	9.39	11.3	35.5	33.0	35.4	4.14	2.63	61.4	45.1

R.B.C. = red blood cells. Hb = hemoglobin (15.6 grams = 100 per cent). M.C.H.C. = mean corpuscular hemoglobin concentration. V_o = mean corpuscular volume. D_o = mean corpuscular diameter. T_o = mean corpuscular thickness = $\frac{4V_o}{\pi(D_o)^2}$. S_o = mean corpuscular surface = $\pi D_o T_o + \frac{\pi(D_o)^2}{2}$.

hypotonic salt solution, so that a 1 per cent solution of sodium chloride could be considered approximately isotonic with undiluted oxalated plasma.

In figure 2 are plotted the results of calculations of the value

$$\frac{V_s - V_o}{V_o}$$

based on data obtained from individual observations on the erythrocytes of 37 blood samples. Each value is plotted as ordinate against the relative osmotic pressure observed to produce 50 per cent hemolysis of the particular sample of erythrocytes as abscissa. Inspection of the points

shown in figure 2 suggests that they fall in general along the extrapolation of the observed curve of percentage increase in volume, which is identical with that shown as a solid line in figure 1. In table 3 the various types

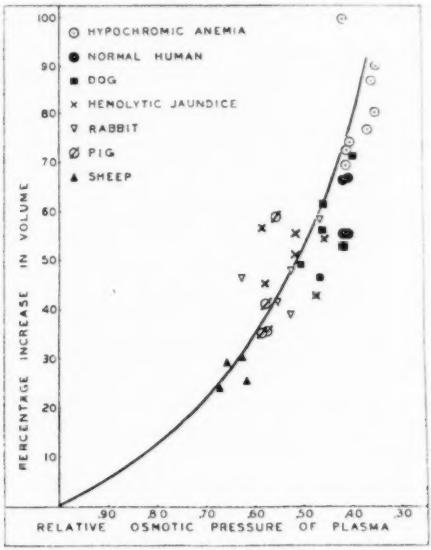

Fig. 2. Percentage increase of discoidal volumes of individual erythrocyte samples calculated from formula

$$\frac{V_s - V_o}{V_o},$$

compared with percentage increase in discoidal volumes at relative osmotic pressures for mean hemolysis, derived by extrapolation of the average curve for equilibrium volumes shown in figure 1.

of erythrocytes are arranged in the order of increasing susceptibility to hypotonic hemolysis. This order was paralleled by the values of

$$\frac{V_s - V_o}{V_o}$$

for each of the various types of erythrocytes and except for the normal human erythrocyte the numerical correspondence with the percentage increase in equilibrium volume, V_h, at the relative osmotic pressure for mean hypotonic hemolysis, P_h, was reasonably good.

Correlation of apparent differences in erythrocyte hemolysis times with differences in discoidal form. Jacobs (6) has presented data on the time

required for 75 per cent hemolysis of erythrocytes of several types of animals following the sudden mixing of a small amount of defibrinated blood with a large volume of 0.02 M sodium chloride solution. On the

TABLE 3

Calculated average percentage increase in volume at relative osmotic pressure for mean hypotonic hemolysis

TYPE OF ERYTHROCYTE	VALUES RELATIVE TO SHEEP ERYTHROCYTE AS FUNCTION OF				ABSOLUTE VALUES		P_h
	V_o	D_o	S_o	$D_o:T_o$	$\frac{V_s - V_o}{V_o}$	V_h	
Hypochromic anemia	54.0	51.0	55.6	82.2	80.3	86.0	0.363
Normal human	72.6	51.9	63.1	64.2	59.5	75.0	0.395
Dog	56.0	46.7	52.1	61.4	57.1	62.3	0.440
Hemolytic jaundice	71.6	49.7	60.6	57.3	51.7	48.0	0.505
Rabbit	60.1	45.0	52.3	50.9	46.1	43.4	0.530
Pig	43.8	39.5	41.6	46.8	42.9	38.5	0.560
Sheep	30.0	30.0	30.0	30.0	27.4	30.0	0.620

V_o, D_o, T_o and S_o are the discoidal volume, diameter, thickness and surface, respectively, of the erythrocytes in isotonic plasma. For convenience, the reciprocal of the $T:D$ ratio of Haden (5) has been used.

$V_h = \left[\frac{1(100 - 51)}{P_h} + 51\right] - 100$ = percentage increase in discoidal volume at P_h.

P_h = relative osmotic pressure for mean hypotonic hemolysis.

TABLE 4

Time required for 75 per cent hemolysis of various types of erythrocytes in 0.02 M sodium chloride

SPECIES	OBSERVED TIME*	CALCULATED TIME	
		$k = 0.3758 \times 10^{-4}$	$k = 0.39 \times 10^{-4}$
	seconds	*seconds*	*seconds*
Man	8.35	**8.35**	6.56
Dog	6.1	5.15	4.76
Rabbit	3.0	3.53	3.40
Pig	3.0	2.75	2.68
Sheep	1.9	1.91	**1.90**

* From M. H. Jacobs (6). The figures in heavy type are the basis for the calculations of the respective values of k.

assumption that differences in such times were due, not to differences in permeability, but to differences in the value of

$$\frac{V_s - V_o}{V_o}$$

it was considered that a relationship to the increase in volume possible without change in surface might be apparent. Jacobs' studies included erythrocytes for which we have pertinent data, namely, those of man, dog, rabbit, pig and sheep. The respective times observed by Jacobs for 75 per cent hemolysis of the erythrocytes of these species at 20°C. are given in table 4.

From these data, according to Jacobs' formula for the rate of entry of water,

$$k = \frac{p_o V_o}{P^2 A t} \ln \frac{p_o V_o - P V_o}{p_o V_o - P V_s} - \frac{V_s - V_o}{P A t},$$

the permeability constants of the human and the sheep cell, respectively, were calculated, utilizing our values for the cell dimensions. In Jacobs' formula, k is the permeability constant; t is the time in seconds required for 75 per cent hemolysis; A is the constant surface of the erythrocyte; V_o and p_o are the initial volume and osmotic pressure, respectively, of the erythrocyte in defibrinated blood (considered isotonic with 1 per cent solution of sodium chloride); V_s and P are, respectively, the spherical volume and the constant external osmotic pressure (0.02 M sodium chloride). Determination of the value of k for the human cell gave 0.3758×10^{-4}, and for the sheep cell 0.39×10^{-4}. Using each value, the theoretical times required for hemolysis of the other types of erythrocytes were then determined by means of Jacobs' formula and are shown in table 4 for comparison with the actual times observed by Jacobs.

Inspection of the data given in table 4 suggests that the theoretical values are at least of the order of magnitude to be expected on the basis of the assumption that differences in shape may account for differences in the time required for hemolysis. Jacobs' observations, as well as our own, are merely averages for each type of erythrocyte. Better correspondence might have resulted had the experiments been made by one observer on the same samples of each type of blood.

Discussion. The data in table 1 suggest that the percentage increases in volume of erythrocytes of different susceptibilities to hypotonic hemolysis are not significantly different. Nevertheless, the question arises whether, despite appearances to the contrary, sufficient variations exist to account entirely for the differences in susceptibility to hemolysis. Determination was therefore made of the theoretical increases in equilibrium volumes of the various types of erythrocytes relative to the sheep erythrocyte, on the assumption that their increased resistance to hemolysis was due *entirely* to proportionately smaller increases in equilibrium volumes.

The equation used by Lucké and McCutcheon (14),

$$V_e = \frac{P_o(V_o - b)}{P_e} + b,$$

expresses the law of Boyle-van't Hoff as applied to cells containing a non-osmotically active fraction. As applied to our experiments, P_o and V_o stand respectively for the relative osmotic pressure of the undiluted oxalated plasma and the volume occupied by the erythrocytes in such plasma. The constant, b, represents the osmotically inactive volume of the erythrocyte. The theoretical values for V_e and P_e giving the best approximation to the average observed values (see table 1) were obtained by substituting for b a value of 51 per cent of V_o. Using this value, the solid curve shown in figure 1 was plotted and extrapolated beyond the range of the observed values according to the formula

$$V_e = \left[\frac{1\,(100 - 51)}{P_e} + 51\right] - 100.$$

Since the result desired is the percentage increase in volume, the original volume occupied by the erythrocytes, V_o, was taken as 100, and the relative osmotic pressure of the undiluted plasma, P_o, was taken as 1.

Approximately 50 per cent of the sheep corpuscles were found to be hemolyzed in a concentration of salt solution or of oxalated plasma of 0.62 with an apparent increase in volume of 30 per cent, according to the solid curve in figure 1. By substituting for V_e the value of 30, and for P_e the respective values for the relative osmotic pressure causing 50 per cent hemolysis of the various types of erythrocytes (see table 3), their respective theoretical values for b were found by solution of the equation,

$$V_e = \left[\frac{1\,(100 - b)}{P_e} + b\right] - 100.$$

Using the hypothetical values of b so found, illustrative curves for the erythrocytes of hemolytic jaundice ($b = 69.4$) and of hypochromic anemia ($b = 82.9$), respectively, were plotted in broken lines as shown in figure 1. Inspection shows that these theoretical curves fall outside the limits of the observed data given in table 1 and represented by the vertical lines intersecting the solid curve in figure 1. Likewise, from a recent investigation of the osmotic properties of the erythrocytes of four species (sheep, ox, rabbit and man) after conversion into a spherical form without change in volume Ponder (3) concludes that differences in water content and in the extent to which the erythrocytes behave as "perfect osmometers" play only a minor part in determining differences in susceptibility to hemolysis. Ponder's observations, however, were made on erythrocytes converted into a spherical form without change in volume by the addition of lecithin. Consequently, the experimental conditions do not necessarily correspond to those obtained when erythrocytes are freely suspended in hypotonic plasma or salt solution.

Because of the limitations upon exact measurement of the erythrocyte

which have been clearly set forth by Ponder (15), the difficulties of subjecting Gänsslen's hypothesis to quantitative test are obvious. It is fully realized that every step in the calculation of the value of

$$\frac{V_s - V_o}{V_o}$$

depends on approximations imposed by the experimental conditions rather than on accurate measurement of the erythrocyte. Thus values for V_o were obtained in oxalated plasma, whereas values for mean diameter, D_o, were obtained in dried smears. Because the precise shape of the erythrocyte is not known, it is obviously impossible to calculate exactly the surface of the erythrocyte at volume V_o. Therefore, von Boros' (12) assumption of its resemblance to a right cylinder appears to be as satisfactory a method as any of obtaining at least a relative value for mean corpuscular thickness and hence for erythrocyte surface.

A certain degree of correlation between mean susceptibility to hypotonic hemolysis and any measurement of the erythrocyte is to be expected if differences in its form are responsible for differences in susceptibility to hypotonic hemolysis. In table 2 the various types of erythrocytes are arranged in the order of increasing average susceptibility to hemolysis. The correspondence, both in order and numerically, of the values of

$$\frac{V_s - V_o}{V_o}$$

with the average values for the relative osmotic pressure causing mean hemolysis has been pointed out. In table 2 are also shown the theoretical percentage increases in volume of each type of erythrocyte calculated as proportional to the discoidal volume, diameter, surface and the discoidal diameter:thickness ratio,[2] respectively, of each of the other types of erythrocytes relative to those of the sheep erythrocyte. Inspection of table 2 shows that the order is preserved only in respect to the diameter: thickness ratios and for the values of

$$\frac{V_s - V_o}{V_o}$$

of the various types of erythrocytes. On the contrary, in respect to correlations on the basis, respectively, of original volume, diameter and surface, the erythrocytes of hemolytic jaundice and of hypochromic anemia present exceptions to this order which throw weight in favor of the significance of the *form* of the erythrocyte in determining its susceptibility to hypotonic hemolysis.

[2] For convenience, the reciprocal of the thickness:diameter ratio of Haden (5) is used.

It is to be emphasized that, whereas the calculations based on the discoidal diameter:thickness ratio give merely *relative* values here calculated with respect to the average sheep erythrocyte as a base, the expression

$$\frac{V_s - V_o}{V_o}$$

is an attempt at an *absolute* value. Thus, when susceptibility of the erythrocyte to hypotonic hemolysis is regarded as a function of the percentage difference between the original discoidal volume and the volume of a sphere of equal surface, the varied susceptibility to hemolysis of the erythrocytes of these different species and pathological conditions can, to some extent, be anticipated.[3]

CONCLUSIONS

The following results suggest that differences in the susceptibility of various types of erythrocytes to hemolysis by hypotonic salt solution are due largely to differences in form, and not to differences in osmotic behavior.

1. The percentage increases in equilibrium volumes in hypotonic plasma of erythrocytes of widely different susceptibilities to hemolysis do not show significant differences.

2. Direct microscopic observations indicate that: *a*. Hemolysis of a given type of erythrocyte is associated with the assumption of a spherical form in hypotonic plasma. *b*. The more susceptible the erythrocyte to hypotonic hemolysis, the less hypotonic is the plasma necessary to cause the assumption of a spherical form.

3. An approximation to the percentage increase in volume necessary to cause hemolysis can be made by calculation of the percentage difference between the volume of the erythrocyte in isotonic plasma, V_o, and that of a sphere of equal surface, V_s.

4. In correlations of the *relative* degree of susceptibility to hypotonic hemolysis of a series of types of erythrocytes with their respective volume, diameter or surface in isotonic plasma, the erythrocytes of hypochromic anemia and of chronic hemolytic jaundice present exceptions.

5. When such correlations are made on the basis of the diameter:thickness ratio or when *absolute* values are calculated according to the formula,

$$\frac{V_s - V_o}{V_o},$$

the erythrocytes of hypochromic anemia and of chronic hemolytic jaundice do not present such exceptions.

[3] Since this manuscript was submitted, similar conclusions have been published by Ponder (16).

6. Differences in the time necessary to cause hypotonic hemolysis of different types of erythrocytes may possibly be explained by differences in the percentage increase in volume of the discoidal form necessary to produce the spherical form in hypotonic solution.

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THE ELECTRIC RESPONSES OF THE FACIAL MUSCLES

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In the course of observations on the changes of the electric responses of cat's skeletal muscles in various experimental conditions (Rosenblueth and Morison, 1937) it appeared desirable to record only from a few units, in order to simplify the analysis of the results. The suggestion arose, therefore, to use localizing lead-off electrodes on the facial muscles, in which a fine layer of fibers is naturally spread out beneath the skin, and to stimulate them indirectly, through the facial nerve. When this suggestion was applied unexpected results were encountered, with which this report is concerned.

METHOD. Cats were anesthetized with dial (Ciba, 0.7 to 0.8 cc. intraperitoneally). A tracheal cannula was inserted. Bipolar or tripolar shielded silver stimulating electrodes were placed on one or more of the several branches of the VIIth nerve at its exit from the cranium. The whole nerve was crushed centrally. When the bipolar electrodes were employed the cathode was toward the muscle; when the tripolar electrodes were used the middle one was the anode, and the two external wires were connected with the cathode of the stimulating circuit. The symmetrical field obtained with this arrangement satisfactorily decreased the stimulus artifacts in the records.

For single shocks condenser discharges (capacity 0.002 to 0.2 μF) were usually employed, and for repetitive stimulation the directly amplified rectangular waves from a photo-electric cell, illuminated at the desired frequency through slits cut in a rotating disk, were passed through a transformer. Exceptionally, induction shocks were applied.

The lead-off electrodes transmitting the responses to the amplifier were silver-silver chloride needles or concentric electrodes (Adrian-Bronk). The amplifier was that used in the previous study (Rosenblueth and Morison, 1937). The amplified signals were photographed from a cathode-ray oscillograph.

Several of the facial muscles were observed—facial portion of the platysma, orbicularis oculi, occipitalis, auriculo-labialis, and others of the auricular group (cf. Huber and Hughson, 1926). They all yielded similar results, with only slight quantitative differences. The contractions were

usually physiological—i.e., neither isotonic nor isometric, the muscles maintaining their normal insertions and the skin being free to move. In some instances, however, the occipital muscle, which is easy to dissect, was exposed; fine bundles of fibers could then be separated and studied by themselves. Nearly isometric contractions of the auriculo-labialis muscle were obtained by fixing the ear and the angle of the mouth so that the skin between them was tightly stretched; the results were identical with those which appeared without these precautions and the point was therefore not further labored.

Results. A single nerve volley elicited consistently complex electrograms in which many independent spikes could be recognized (see fig. 3). The spikes were more or less clearly separated, depending on the lead-off

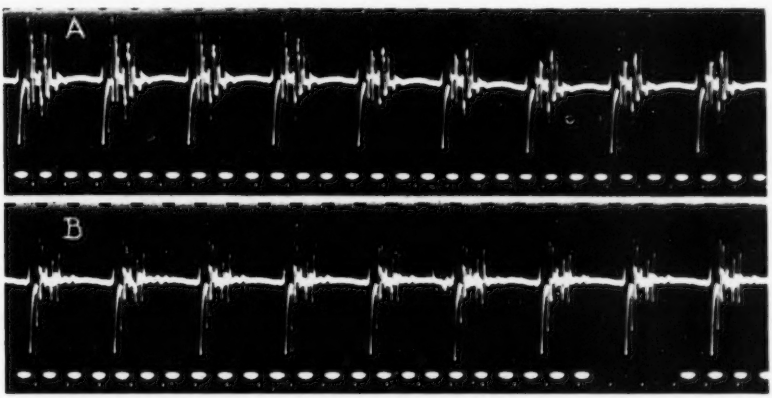

Fig. 1. Platysma (near mouth). Isometric contraction. Beginning (A) and end (B) of a series of maximal stimuli at the rate of 30 per second. In this and the succeeding figures the electric responses were recorded through concentric electrodes of the Adrian-Bronk type. The small intervals in the time signal denote 10 msec.

electrodes employed. The relatively localizing concentric leads yielded distinguishable individual spikes. The less discriminating silver needles, placed several centimeters apart, resulted in a tendency for the appearance of a single prolonged, largely fused wave, although its composite nature was usually recognizable.

The latency of the first spike in the responses varied with the muscle studied and the position of the recording electrodes. From the middle of the auriculo-labialis muscle it was about 2.5 msec. The latency of the last spike in an unpotentiated response (see below) to a single volley varied in eight experiments on the auriculo-labialis from 10 to 32 msec. (average 20), and in six experiments on the occipitalis it varied from 11 to 22 msec. (average 18).

On tetanic stimulation at a frequency of 30 or 60 per sec., patterns similar to that occurring after the first shock recurred with uncanny regularity. Figure 1A shows the beginning of a series at 30 per sec. The individual spikes are clearly recognizable for the successive shocks throughout the series. Even 20 seconds later (fig. 1B) the pattern per shock bears a striking similarity to the early responses.

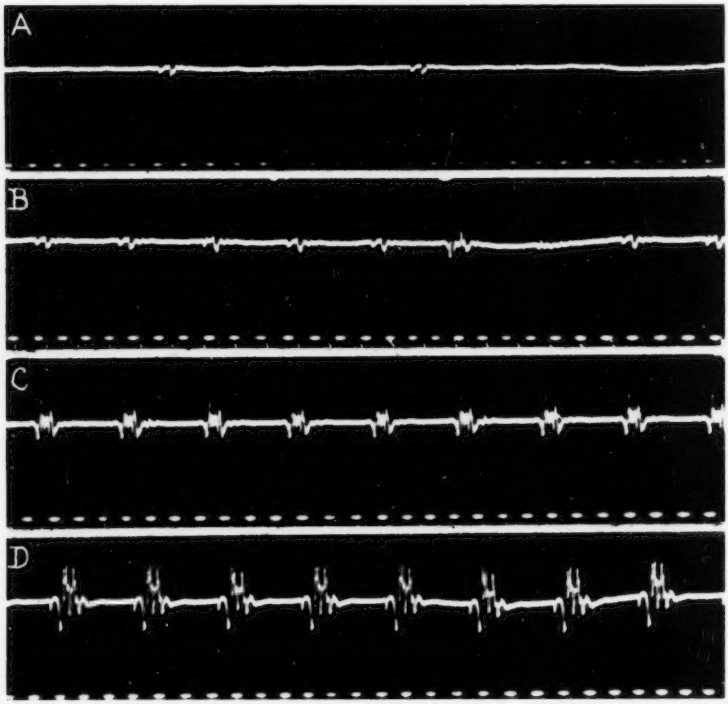

Fig. 2. Auriculo-labialis. Stimulation at the rate of 30 per second. The intensity of the stimuli was progressively increased from A, where it was just threshold, to D, where it was maximal.

Careful measurement of the latencies of some of the individual spikes in a tetanus consistently showed systematic changes through the period of stimulation. The latencies first decreased—as much as 1 msec. less than in the first or second shock; they later increased, usually to the original values, sometimes as much as 0.5 msec. more than in the first shocks.

At higher frequencies of stimulation, e.g., at 120 per sec., the responses were at first quite regular; after some seconds (e.g., 5 to 10) alternation and simplification occurred. In this simplification there was no systematic

tendency of the most delayed spikes to disappear first. As a rule such a progressive disappearance was random, and occasionally some of the early spikes dropped out almost entirely while some of the delayed excursions were practically unchanged.

When just threshold shocks were applied to the nerve the first spikes to occur were as a rule not those with the shortest latency, but some with an intermediate delay. As the intensity of the stimuli was increased the spikes already present sometimes apparently increased in size, sometimes were unchanged, while new spikes occurred, both earlier and later than the first obtained. Figure 2 illustrates a typical instance.

The influence of a previous tetanic stimulation on subsequent single maximal shocks (cf. Schiff, 1858) was tested in 5 animals. In 2 of these there were no changes or even slight decreases in the magnitude and number of spikes in the responses to single shocks. In the other 3 an increase occurred. The lack of rigid fixation of the muscles might easily lead to slight changes in the position of the electrodes during the tetanus, which

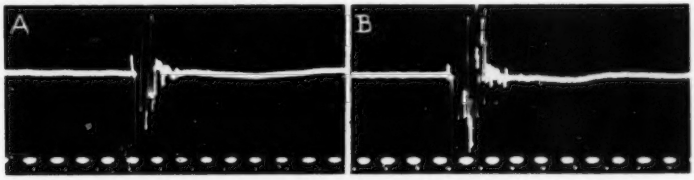

Fig. 3. Auriculo-labialis. Responses to single maximal shocks. A, before, and B, 10 seconds after tetanizing for 30 seconds at the rate of 60 per second.

could modify the response recorded. The increased responses in two of the experiments were so long, however (up to 80 msec.), as to render it quite unlikely that a shift of the electrodes could be responsible for the increment, for these responses were much longer than any obtained without a previous tetanus. Figure 3 illustrates one of the positive experiments—not the extreme instance. The post-tetanic increment lasted for several minutes.

Injections of prostigmin led to a marked increase in the duration of the electrograms when single shocks were applied to the nerve. Repetitive stimulation (30–60 per sec.) after prostigmin resulted in a decline of the responses per shock, which with high frequencies (60–120 per sec.) was practically complete (cf. Rosenblueth and Morison, 1937).

Injections of small doses of curare produced a reduction of the electrograms (fig. 4A, B and C). This reduction was manifest either as a decrease of the amplitude of some of the spikes or as a disappearance of some, or both. No systematic tendency for the disappearance of the several spikes occurred; the first, middle or last could be the earliest abolished. Larger doses of curare cancelled all responses. The muscles are relatively resistant

to the drug; in some animals respiration had been completely paralyzed and some electric responses of the facial muscles were still present.

Repetitive stimulation after intermediate doses of curare resulted in a decline of the electrograms. A parallel decline of the mechanical responses was also observed—i.e., Wedensky inhibition (cf. Hoffman, 1903). Even frequencies of stimulation as slow as 1 per sec. produced clear reductions; more marked effects were obtained, however, with higher frequencies (fig. 5).

Tetanic stimulation after intermediate or completely paralyzing doses of curare caused a subsequent increase or reappearance of the responses to single shocks for some seconds or minutes (fig. 4D, E and F). This post-

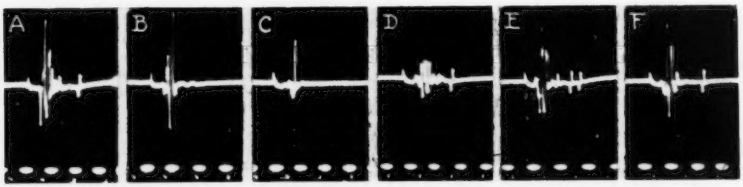

Fig. 4. Auriculo-labialis. Responses to single maximal shocks. A. Before curare. B. After a small dose of curare. C. After a further small dose. D. Immediately after tetanizing for 10 seconds at the rate of 120 per second. E. and F. 30 and 60 seconds later.

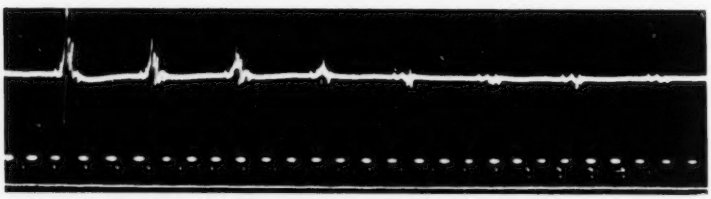

Fig. 5. Auriculo-labialis. A small dose of curare was injected. Responses to a series of maximal shocks at the rate of 30 per second.

tetanic decurarization is similar to that described by Boyd (1932) for the tongue muscles. The position of the spikes on such a decurarization was random—short or long latencies appeared indiscriminately.

Direct stimulation of the occipitalis muscle, or of a dissected portion of it, was made before and after curare. Without curare, the shortest latency of the responses obtained varied with the position of the stimulating electrodes. It was sometimes short (about 1.5 msec.) and the records were then quite similar to those obtained from indirect stimulation. In these cases the muscle could contract almost over its entire length. Other positions of the stimulating electrodes yielded only localized responses, which did not extend more than 1.3 cm. beyond the electrodes. The latency was

then long (about 8 msec. with a lead-off distance of 1 cm.). These records were as a rule simpler than those from indirect activation, consisting of fewer spikes or even of a single smooth wave. After complete curarization similar limited contractions invariably occurred. The measurements of the extent of contraction made by direct observation were confirmed by the electrical observations: action potentials were recorded only if the lead-off electrodes were less than 1.3 cm. away from the stimulating electrodes. The latencies of the several spikes, when such responses were composite, varied from 8 to 15 msec. for lead-off distance of 1 cm.

Discussion. The complex responses of the facial muscles to single stimuli (fig. 3) could be due either to repetitiveness or to temporal dispersion, or both—i.e., the single stimuli might evoke repetitive nerve discharges, in turn leading to repetitive muscular responses; or else single nerve volleys might evoke repetitive muscular discharges; or, finally, different muscle units or fibers might discharge only once but with large asynchronism.

All the data reported clearly indicate that the records obtained without the increasing action of a previous tetanus or of prostigmin are dispersed, not repetitive, responses. Thus, if the responses were repetitive the spikes which would disappear earlier on tetanic stimulation or after curare would be the later ones and the first spike of the response would be the more persistent. This was not found to be the case (figs. 4 and 5). When threshold shocks were applied, if the responses were repetitive, the first spike should be the earliest to appear, which again was not the case (fig. 2). Furthermore, the action potentials of the nerve were recorded in two experiments and single shocks elicited typical single volleys. Finally, the intervals at which the first spikes in a response follow one another may be so short (1 msec. or less) as to exclude repetitive discharges in the same fibers.

It can be concluded, therefore, that a high degree of temporal dispersion occurs in the facial muscles. In other muscles studied there is also temporal dispersion, but to a lesser extent. Thus in the frog's sartorius, according to Schaefer's (1936) data, the dispersion cannot be greater than 3 msec., since the total duration of a maximal spike was only 4 msec. In our records of cat's tibialis anticus and gastrocnemius (Rosenblueth and Morison, 1937) the dispersion was usually not greater than 10 msec., judged by the same criterion.

How may such prolonged dispersions be accounted for? The conduction distance in the nerve was not greater than 6 cm. in any of the observations. The motor fibers are mainly A fibers, of high conduction velocity. The two experiments in which the nerve action potentials were recorded did not show a significant scattering of the spike potentials at a distance of about 2 cm. from the stimulating electrodes. It may be concluded, therefore, that temporal dispersion in the arrival of the nerve impulses at the muscles could

probably account for only a minor fraction of the observed differences in latency.

No data are available, apparently, on conduction rates of mammalian muscle fibers. The present observations on direct stimulation of normal and curarized muscles (p. 389) indicate conduction rates of from 0.6 to 1.2 m. per sec. for the fibers of the occipitalis. These figures are probably low, because the muscle was exposed in those experiments, and therefore probably cool.

The length of the longest fibers of the occipitalis, according to the data reported (p. 388), is about 1.3 cm. This figure agrees with those determined by anatomical methods for other similar muscles (see Häggqvist, 1931, for references). Since the nerves join the muscle fibers at about the middle, the maximum length of conduction in a fiber would be about 7 mm.

Even if the figures calculated above for conduction rates should be approximately correct, different rates of conduction in different lengths of muscle fibers could only account for 12 msec. of temporal dispersion at the most. There would still remain at least 6 msec. to be explained (see p. 385).

The only remaining factor which can account for the prolonged latencies occurring in some of the fibers is the neuromuscular delay. The previous measurements of this delay have usually dealt with the shortest one present. No data are available as to how much it may vary from one fiber to another and how long it may be. The present data favor the view that the range of distribution of neuromuscular delays may be extensive for the facial muscles and that delays as long as about 10 msec. may occur. That a prolonged neuromuscular delay can be observed in some experimental conditions has been shown by Forbes, Ray and Griffith (1923) and by Hoefer (1933).

Three hypotheses have been suggested to account for the neuromuscular delay of skeletal muscle. The first is that the delay represents the "utilization period" of the nerve action potential stimulating the muscle electrically (cf. Forbes, Ray and Griffith, 1923); this view is untenable for delays which long outlast the action potential. The second is that the delay represents the time necessary for summation of punctate electrical activation (Eccles, 1936); this hypothesis has no experimental support. The third is that the delay represents the time necessary for liberation and mobilization of acetylcholine, the chemical mediator of the motor nerve impulses (Brown, Dale and Feldberg, 1936; cf. Rosenblueth and Morison, 1937); the present data are satisfactorily explained by this view.

In a previous study (Rosenblueth and Morison, 1937) evidence was presented that acetylcholine may accumulate early in a series of frequent repetitive stimuli and that it may become relatively exhausted after prolonged stimulation. The changes of latency in a given group of muscle fibers on repetitive stimulation at a frequency of 30 or 60 per sec. reported

above (p. 386) can be explained on that basis: a remnant of acetylcholine from previous shocks at the beginning of a tetanus would shorten the neuromuscular delay; a decline of the quanta of acetylcholine released per nerve volley after prolonged activation might increase the delay.

The increased responses to single volleys after prostigmin (p. 387) are explicable in the present, as in other instances, by repetitive discharges in the muscle (Brown, Dale and Feldberg, 1936). The depression of the responses on repetitive stimulation after prostigmin (p. 387) is attributable to a paralyzing accumulation of acetylcholine, protected from destruction by the drug (cf. Rosenblueth and Morison, 1937).

Since the total duration of the responses to single maximal volleys may be increased after a tetanus (fig. 3) it can be concluded that repetitive discharges of some muscle fibers occur in these circumstances. This conclusion was tentatively suggested for similar observations on the gastrocnemius muscle (Rosenblueth and Morison, 1937). The long duration of the post-tetanic decurarization (fig. 4) reveals an enduring effect of the motor nerve impulses, even when they do not lead to contraction. The nature of this effect is obscure; further studies are necessary for its elucidation.

SUMMARY

The electric responses of the facial musculature were studied in cats. Single shocks applied to the facial nerve elicit complex effects in which many spikes of different latencies are visible (fig. 3).

The effects of repetitive stimulation (fig. 1), of varying the intensity of the stimuli (fig. 2) and of curare (figs. 4 and 5) lead to the conclusion that the complex records obtained are due to temporal dispersion (p. 389). The variable latencies for different fibers in a muscle are mainly due to variable neuromuscular delays (p. 390).

Several theories for the neuromuscular delay are discussed (p. 390). It is concluded that the most acceptable explanation is that the delay is due to the time necessary for the liberation of acetylcholine—the chemical transmitter of the motor-nerve impulses (pp. 390–391).

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PHYSIOLOGICAL PROPERTIES OF A CENTRAL EXCITATORY AGENT IN FLUID OBTAINED BY OCCIPITAL PUNCTURE OF MAN AND ANIMALS

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Extracts of fluid obtained by occipital puncture in man have been reported to cause marked rise in arterial pressure when injected into cats which were lightly anesthetized and with their vagi severed (Page, 1935). The pressor action was not observed when the central nervous system was destroyed, or when its function was impaired. Consequently, it was concluded that the nervous system was an essential link in the pressor mechanism. Further studies on the physiological properties of these extracts have been made and are reported in this communication.

Since such extracts stimulate other nuclei in the brain than those concerned with the pressor mechanism, such for example as the vagus nuclei, it seems desirable to call the active substance (or substances) central excitatory. For convenience then, the active material in extracts of fluid from the cisterna magna and the third ventricle will be called a "central excitatory agent" (abbreviated C. E. A. in this communication). By this connotation it is meant to avoid any implication that it is physiologically active.

METHOD. The method employed to study the action of extracts was the same as that previously described (1935). In essence it consisted in adding the fluid to be tested to alcohol in the proportion of 1 to 50, filtering off precipitated protein after it has stood in an ice box, and removing the alcohol under reduced pressure. Removal of the alcohol usually caused the volume of the original fluid to be reduced to one-half, but not more and *usually less.* The extracts were water-clear and gave no precipitate with 95 per cent alcohol. This aqueous residue was injected intravenously into cats. The animals were prepared by lightly anesthetizing with ethyl urethane and four to five hours later both vagi were severed. Arterial pressure was recorded by a mercury manometer from the right carotid or femoral artery. Heparin was used as anticoagulant in the cannula and in the tubing connecting it with the manometer. The effect of the volume of the injection was estimated by injecting the same amount of warm physiological saline solution before making the test injection. Usually the

volume was less than 2 cc.; consequently, it did not affect the blood pressure significantly.

Fluid was obtained from patients by occipital puncture within as short a period after death as possible.[1] This varied from 10 minutes to 24 hours. Fluid was also obtained from the third ventricle and cisterna magna after the brain had been opened. Occipital punctures were performed also on living animals and the first 1 to 2 cc. of fluid employed. Some of the dogs had normal blood pressures, and some had been made hypertensive by

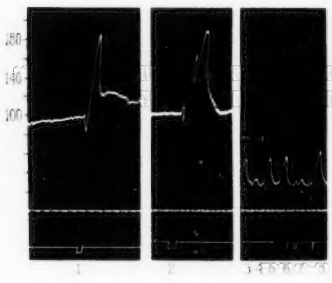

Fig. 1

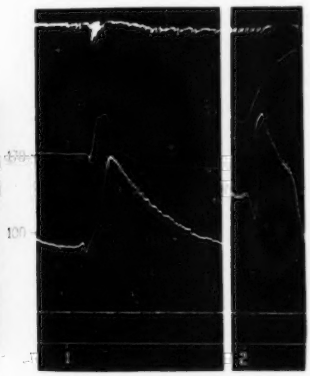

Fig. 2

Fig. 1. Pressor effect of cisternal and spinal fluid extracts from patient suffering from malignant hypertension (B. P. = 230/176). *1*. Extract of 0.5 cc. of fluid obtained by occipital puncture. *2*. Alcohol extract of 1.2 cc. spinal fluid. *3*. Pithed cat; 1 cc. of 1:100,000 adrenalin. *4*. Alcohol extract of 3 cc. of spinal fluid. *5*. Adrenalin. *6*. Adrenalin. *7*. Extract of 5 cc. spinal fluid. *8*. Adrenalin. Time marker represents base line. Each grouping of 10 vertical lines represents 1 minute.

Fig. 2. Effect of extract of occipital fluid of a normal anesthetized dog on blood pressure and nictitating membrane of an adrenalectomized cat injected with cocaine (4 mgm.). Upper line represents respiration; middle line the movements of the nictitating membrane; lower line of the arterial pressure. At *1* and *2* extract of 1.5 cc. of occipital fluid was injected.

constricting the renal arteries (method of Goldblatt, Lynch, Hanzal and Summerville, 1934).

Results. *Effect on anesthetized cats with both vagus nerves severed. Character of the pressor response.* The pressor response was initiated usually by a slight fall in blood pressure which was followed by a sharp rise about 10 seconds after completion of the injection. During the ascent,

[1] I wish to express my appreciation to Dr. Milton Helpern of Bellevue Hospital for his aid in obtaining some of these fluids from cadavers and to Dr. Charles Elsberg and Dr. Byron Stookey for fluid from the lateral ventricles of patients.

the pressure might reach a plateau followed again by a still higher rise. It then fell in from 2 to 3 minutes to the original level. Less frequently it required 4 to 16 minutes, or more, to return to normal. The plateau in the curve might not occur, nor was the preliminary short fall in pressure always seen.

Effect on nictitating membrane. C. E. A. caused contraction of the nictitating membrane in cats (fig. 2). Contraction often occurred even when arterial pressure fell instead of rising. But the extent of contraction was usually not as great as that elicited by injection of epinephrine. It was much less than the powerful contraction of the membrane produced by tyramine.

Sensitization of the nictitating membrane to epinephrine was secured in two ways, namely, denervation by removal of the superior cervical ganglion (Meltzer and Auer, 1904) and by injection of cocaine (Rosenblueth and Cannon, 1932).

The membranes were found very sensitive to epinephrine eight days after denervation, but C. E. A. failed to cause immediate contraction. When the arterial pressure fell, or toward the end of the pressor response, a small contraction of the membrane was obtained. It was believed that this was due to secreted epinephrine. Membranes sensitized by intravenous injection of cocaine responded moderately to stimulation by C. E. A. but the response was less marked than before the injection of cocaine.

Repeated injections of C. E. A. caused the normal membrane to respond more and more sluggishly until it ceased to respond altogether. Injection of epinephrine or tyramine at this time still caused a sharp contraction of the membrane.

In two experiments it was found when ergotoxin had reversed the pressor action of epinephrine on arterial pressure to depressor, and the membrane still contracted, that C. E. A. no longer caused contraction. This action is possibly due to toxic action of the drug on the central nervous system.

The facts that C. E. A. ceased to stimulate contraction when epinephrine and tyramine still caused powerful contraction, that denervation abolished the response, that cocaine decreased rather than increased the sensitivity of the membrane, and that the membrane was so easily "fatigued" by C. E. A., suggested that stimulation of it was mediated through the nervous system. This contrasted with the direct action of epinephrine and tyramine.

Certain effects on respiration, renal volume and the eyes. The response of respiration to C. E. A. varied greatly in the same and in different cats. Often there was immediate increase in rate and depth followed by a short period of standstill. The increase might not appear and about 30 seconds after the maximum rise in arterial pressure had occurred the respiration became shallower until standstill occurs, followed in another 30 seconds by resump-

tion of the original rate and depth. Still another common type of response was marked increase in rate and depth without respiratory standstill. There appeared to be no correlation between the respiratory response and the response of arterial pressure.

Four experiments were performed in which the right kidney of the cat was placed in a Livingston oncometer connected with Brodie bellows. Rise in arterial pressure was usually accompanied by sharp reduction of renal volume, but even if a fall in pressure resulted from the extract, renal volume was reduced. Removal of the kidneys, intestine, liver, adrenals, spleen and pancreas did not, however, prevent a rise in arterial pressure by C. E. A. (2 experiments).

It was often noticed that when the response to C. E. A. was powerful the eyes of the anesthetized animal appeared to bulge from their sockets and extreme miosis occurred. Both quickly subsided.

Effect of C. E. A. on blood pressure and heart rate before and after section of the vagus nerves. When C. E. A. is injected before the vagus nerves are severed, a transient and, at times, prolonged fall in blood pressure occurs. The heart rate is sharply reduced. If the pressure is allowed to return to its original level and then the vagi are severed quickly, the arterial pressure rises rapidly, often as much as 120 mm. Hg. The rate of the heart beats increases markedly. For example, in one experiment the blood pressure fell 74 mm. Hg. and the heart rate decreased 60 beats per minute. As the rise in pressure to its original level occurred both vagus nerves were cut. The blood pressure rose 154 mm. Hg and the heart rate increased 100 beats per minute.

The same experiment has been repeated four times using histamine to reduce the blood pressure. In two of the experiments, when the pressure was reduced 92 and 96 mm. Hg respectively, no change in heart rate occurred. Section of the vagi caused increases of 37 and 90 beats per minute. In the third experiment the heart rate increased 12 beats per minute while the blood pressure fell 52 mm. Hg; the rate then rose 30 beats after section of the vagi. In the fourth experiment, the heart rate fell 40 beats with a concomitant fall of 72 mm. Hg in arterial pressure. The rise in pressure after vagus section was less marked when histamine was used to alter the blood pressure than with C. E. A.

If, after the return of pressure to normal levels following section of the vagi, more C. E. A. is injected, only a slight, if any, fall in pressure occurs, and the blood pressure rises sharply and markedly. The heart rate is usually not greatly changed even at the peak of the pressure. In most of the experiments, a rise of from 2 to 30 beats occurs.

When the vagi are intact, they restrain markedly the rise in arterial pressure which follows injection of C. E. A. In some experiments this restraint appears to be so great that only a moderate rise occurs after a

period of reduced pressure and slow heart rate. Liberation from the restraint due to the vagi is followed by a sharp rise in pressure. From these experiments it is concluded that the nuclei of the vagus nerves are strongly stimulated by C. E. A. and that these nerves act to restrain the simultaneous sympathetic stimulation. The carotid sinus mechanism does not appear to play a predominant part in the inhibition because in three experiments the blood pressure rose sharply when, prior to the injection, both vagi were cut and both carotid arteries tied off above and below the sinus. If, however, the vagi were left intact and both carotid arteries tied off, vagus inhibition occurred as when the arteries were not occluded. It is not meant to imply that the carotid sinus mechanism does not influence the response to C. E. A. but simply that response may occur even when it is inactivated.

Effect of destruction or reduction of the function of the nervous system. After it had been shown that the cat used for the experiment responded actively to C. E. A., the carotid arteries were tied off and it was quickly pithed through a trephine hole in the skull thus destroying brain and most of the cord. Time was allowed until the blood pressure had assumed a constant level and the pressure had been shown to respond to small doses of epinephrine. The same amount of C. E. A. employed before pithing was then injected. It was invariably found that no noteworthy response was obtained (fig. 1).

Reduction of nervous function by anesthesia also prevented the response. If the animals were so deeply anesthetized that they either did not respond, or responded only sluggishly to inhalation of 10 per cent CO_2-air mixture, or to stimulation of the carotid sinuses by occlusion of the common carotid arteries, the response to C. E. A. was either minimal or failed entirely. For this reason, failure to obtain a response does not always indicate that the extract is inactive. Experience has taught that the best time for obtaining active responses is from 4 to 5 hours after intraperitoneal injection of 6 cc. of a 25 per cent solution per kilogram of ethyl urethane.

The effect of anesthesia on the C. E. A. response is similar to the effect on the response of repeated injections of C. E. A.

Successive injections of C. E. A., even if ample time was allowed for the blood pressure to return to the pre-injection level, was followed rapidly by progressively weaker responses, until not only is the pressor action abolished, but sharp depression of arterial pressure occurs. Cats were occasionally found that responded to C. E. A. only by reduction of the pressure level. Quantitative assay by this method was consequently very difficult (fig. 3).

Effect of adrenalectomy and splanchnic resection on the pressor response. The occurrence of a plateau in the curve of arterial pressure suggested that after the initial rise the secondary one was due to secretion of stimulated

adrenal glands. Bilateral adrenalectomy using the posterior approach was performed both one hour before the injections were made and directly after a pressor response had been elicited. Seven experiments were carried out (fig. 4). The operation usually modified the response somewhat in that the rise in pressure was not as marked. In two experiments, the same extracts which had caused rise in pressure caused a sharp fall after adrenalectomy. However, in most experiments, the pressor response was still obtained and even the plateau was observed. While adrenalectomy appeared to reduce the intensity of the pressor response, it did not abolish it, and consequently could not be held responsible for the chief action of the

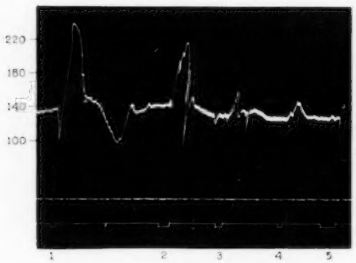

Fig. 3

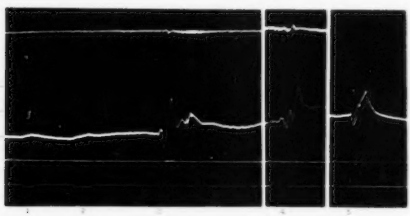

Fig. 4

Fig. 3. Effect on cats' blood pressure of repeated injections of extract of ventricular fluid from patient with chronic nephritis and hypertension (Hosp. no. 8295). *1.* Extract equivalent to 2 cc. of ventricular fluid. *2.* Same, equivalent to 3 cc. *3.* Same, equivalent to 2 cc. *4.* Same, equivalent to 2 cc. *5.* Same equivalent to 4 cc. ventricular fluid.

Fig. 4. Effect of suprasellar cyst and ventricular fluid extract from a patient suffering from a craniopharyngioma on cats' arterial pressure. *1.* Five cubic centimeter of saline. *2.* Ten cubic centimeters of fresh ventricular fluid. *3.* Extract of 0.5 cc. of fluid from the cyst. *4.* Extract of 3 cc. of ventricular fluid. *5.* Extract of 1 cc. of cyst fluid injected after bilateral adrenalectomy. The upper line represents respiration.

extracts. To this evidence must be added the fact that pithing abolishes the response completely. If it were assumed that C. E. A. had a direct action on the adrenal glands, this operation should not interfere with the response. These experiments suggest that, although the character of the blood pressure response is somewhat altered by removing the adrenal glands, they are not necessary for it.

In one experiment the splanchnic nerves were resected just below the diaphragm, partially denervating the adrenal glands. The response to C. E. A. was as great after the operation as before. This emphasizes again that the adrenal glands are not essential for the pressor response.

Effect of ergotoxin,[2] *cocain, and atropin on pressor response.* Were the

[2] Ergotamine tartrate-Sandoz Chemical Company.

pressor action due to liberation of epinephrin, it might be expected that ergotoxin would reverse it from pressor to depressor, just as is observed when epinephrine is injected (Dale, 1906). Experiments were performed in which sufficient ergotamine tartrate was given to cause sharp depression of arterial pressure when epinephrin was injected in doses which prior to the ergotamine produced a rise in pressure. The response to C. E. A. was often reduced in intensity but reversal did not occur. Diminution in magnitude of the response may be only partially due to ergotamine because the second dose of extract always produces a less active response than the first (fig. 5, nos. 3 and 6, and fig. 5, nos. 6 and 11).

Atropin in doses sufficient to reverse the depressor action of choline to pressor also did not appear to have any characteristic effect on the response to C. E. A. (fig. 5, nos. 8 to 11 for example.)

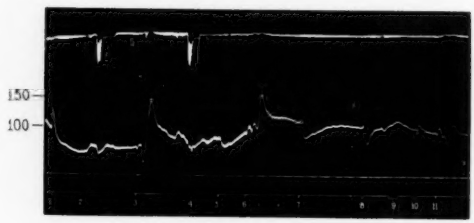

Fig. 5. Effect of ergotoxin and atropin on pressor action of extract of occipital fluid of dogs. *1*. Adrenalin 0.5 cc. of 1:100,000. *2*. Saline 2 cc.—animal struggled. *3*. Extract of fluid from normal dog 0.5 cc. *4*. Ergotamine tartrate 1 cc. of 1:2000 solution. *5*. Adrenalin. *6*. Same extract as (*3*). *7*. Adrenalin. *8*. Choline 1.5 mgm. *9*. Atropin grain 1/50. *10*. Choline 1.5 mgm. *11*. Same extract as *3*, upper line represents respiration.

Cocaine, as shown by Fröhlich and Loewi (1910) produces marked potentiation of the pressor action of epinephrin. After such potentiation had been secured and a dose of C. E. A. had been given as a control, the same C. E. A. was retested. It was found that cocaine had no effect on the response. Occasionally, it was observed that the response was reduced. This was believed to be due to the toxic action of cocaine on the nervous system, as in some of these experiments other signs of intoxication were noted such as irregular rhythm of the heart.

Comparison of epinephrin and C. E. A. on arterial pressure. A series of 4 experiments have been performed to ascertain whether the response to C. E. A. varies in some regular fashion with the response to epinephrin. These experiments leave no doubt that parallelism does not exist. A strong pressor response can be elicited by C. E. A. at times when the animal responds by fall of blood pressure to the dose of adrenalin which usually causes a sharp pressor action.

Sources of C. E. A. It may be of interest to mention briefly some of the sources of fluids from which C. E. A. was prepared. Fluid obtained by occipital puncture from patients suffering from a variety of diseases have yielded it. These fluids are by no means limited to patients suffering from hypertension. Occipital puncture in dogs both before and after hypophysectomy yield it. Consequently, the hypophysis is not responsible for the genesis of the active substance. C. E. A. may be obtained from occipital fluid from normal cats and goats.[3] Fluid obtained at operation from a suprasellar cyst also yielded it.

Fluid obtained by spinal puncture or from the lateral ventricles of the brain usually yields extracts, in our experience, which contain only very small amounts of C. E. A., comparable in amount to that in blood. Consequently, only the first portion of fluid removed from the cisterna is employed in the preparation of extracts. In cats, for instance, only the first 1 or 2 cc. of fluid contain the active material. Only under circumstances, not as yet clearly defined, does fluid from the lateral ventricles and the spinal canal yield C. E. A. in amounts which appear to be unusual.

Since C. E. A. may be obtained from a variety of animals and men during both normal and morbid states, the substance or substances apparently do not appear alone during disease.

It appears to be partially bound to some substance in the fluid contained in the cistern, because injection of fresh fluid without preliminary treatment with alcohol causes a rise in blood pressure usually less than 20 per cent of the rise when the same fluid is treated with alcohol. Its combination with other substances must be very weak to be broken by such gentle treatment.

SUMMARY

1. Fluid obtained by occipital puncture from dogs before and after hypophysectomy, cats, goats and man, yields an extract which when injected into anesthetized cats produces physiological evidence of marked central excitation. If the vagi are intact the blood pressure falls and the heart is markedly slowed followed later by a rise in pressure and increase in heart rate. If the vagi are severed, injection of C. E. A. (abbreviation for a central excitatory agent) is associated with a sharp rise in blood pressure without significant change in heart rate (fig. 1). Simultaneously exophthalmos, retraction of the nictitating membrane and other evidences of excitation occur.

2. The excitation appears to be mediated by the nervous system because *a*, injury to or removal of the central nervous system abolishes the response; *b*, the response is rapidly fatigued (fig. 3); *c*, adrenalectomy, splanchnic

[3] An extract of 4 cc. of fluid from a goat raised a cat's blood pressure 98 mm. We wish to thank Dr. J. Beard for the occipital fluid of goats.

nerve resection and carotid sinus denervation may modify somewhat but do not inhibit completely the response; *d*, the response differs in several respects to peripherally acting substances such as epinephrin and tyramine.

3. The pressor response is probably not due chiefly to epinephrin secreted by the adrenal glands because *a*, adrenalectomy alters but does not abolish the response (fig. 4); *b*, control periods of asphyxia do not duplicate the response; *c*, cocain does not potentiate the response either of the blood vessels or the nictitating membrane; *d*, ergotoxin in doses sufficient to reverse the pressor response of epinephrin to depressor do not reverse that of C. E. A. (fig. 5); *e*, denervation of the nictitating membrane by superior cervical ganglionectomy abolishes the immediate response to C. E. A. but sensitizes it to epinephrine; *f*, repeated doses of C. E. A. rapidly diminish the response of the mechanism responsible for the rise in arterial pressure contrary to the action of epinephrin (fig. 2).

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STUDIES ON THE SENSITIVITY OF SMOOTH MUSCULATURE TO EXOGENOUS EPINEPHRINE[1]

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In this paper are presented quantitative data on the relative degrees of sensitization of smooth musculature induced by denervation, total hypophysectomy, thyroidectomy, gonadectomy, adrenalectomy and thyroid feeding. Evidence in support of the theory of specificity of epinephrine sensitivity is given. A search for a cytological basis for the sensitivity by the use of micro-incineration and spectography, as well as the more standard histological procedures, is reported on.

MATERIALS AND METHOD. For the experiments dealing with hormonal imbalance cats were utilized. A pupil on one side was sensitized by removal of the superior cervical sympathetic ganglion. After two weeks, an adequate interval as shown by experimental trials, sensitivity curves for the pupil and nictitating membrane to small doses of epinephrine (1 to 1,000 Parke Davis) administered subcutaneously were established by three or four trial tests. Response to such injections was found to be remarkably constant. The parasympathetic supply to the iris was disregarded as others have shown that this effects only a quantitative difference in the results. Then operative procedures for removal of various glands of internal secretion were carried out and the epinephrine response of the sensitized pupil to the same doses as used preoperatively determined. Changes in weight were generally slight if any. When they were of significant amount a compensation in dosage was effected. No special comment concerning the operative technique is warranted other than to note that hypophysectomy was carried out through the mouth with little or no injury to the basal ganglia. The anorexia, so marked a feature of the postoperative course in these animals, was generally overcome by the use of an acid extract of beef anterior pituitary lobe prepared according to the method of Loeb and Bassett (1930).

The alteration of the epinephrine response of normally innervated tissues by endocrine imbalances was tested for by comparing the response of the

[1] This research was made possible by grants-in-aid for research by the American Medical Association and by the National Research Council.

normal pupil and nictitating membrane to larger doses of intravenous epinephrine before and after the initiation of such imbalances. This was necessary because normal tissues showed no evidence of change to doses administered subcutaneously.

Whenever the possibility existed that the nature of the results obtained by the various procedures carried out in the research might rest on an altered absorption due to changes in the response of the blood vessels they were checked by the intravenous route. No qualitative differences were detected. Quantitatively the results may be somewhat modified by the choice of the subcutaneous route for epinephrine injection but it has many advantages over the intravenous route. Among these are lessened excitement on administration, ease of administration, and a more prolonged action.

To investigate the specificity of the sensitization to epinephrine[2] induced by denervation, blood vessels of the posterior extremity of rabbits on one side were sensitized by removing the abdominal sympathetic chain from the diaphragm to the pelvis. At frequent intervals, from two weeks to six months, the blood vessels of the hind legs of animals so prepared were perfused both at a constant pressure and at a constant rate by a modified method for perfusion of blood vessels previously described by Bishop, Heinbecker and O'Leary (1933). The vessels of the opposite normal leg served as a control. Locke's solution with a concentration of epinephrine (1 to 300,000) or pituitrin (1 to 4,000) served as a perfusate.

In the investigation of blood vessels to determine the presence or absence of cytological change as a consequence of denervation, dogs and cats were used.[3] The blood vessels of one posterior extremity were sensitized by removal of the ipsolateral abdominal sympathetic chain from diaphragm to pelvis. At intervals from two weeks to one year animals so prepared were sacrificed and their tissues investigated. The tissues removed were generous portions of the femoral arteries and veins on the two sides from the mid thigh region. These tissues were fixed routinely in Zenker's fluid, absolute alcohol and formalin. In addition portions of the arteries were prepared by the Altman-Gersch frozen dehydration method as modified by Scott and Williams (1934). Frozen sections were made and stained with Scharlach R and sudan III. Tissues fixed in alcohol and formalin and those prepared by the Altman-Gersch method were incinerated according to the technique of Scott (1933). Comparisons in spectograms were made of control and treated vessels to determine what qualitative change in inorganic salts or metals might have taken place. Incinerations were done to ascertain the changes or shifts in salt distribution within the smooth muscle cells of the vessel walls.

[2] This work was done in association with Dr. George H. Bishop.

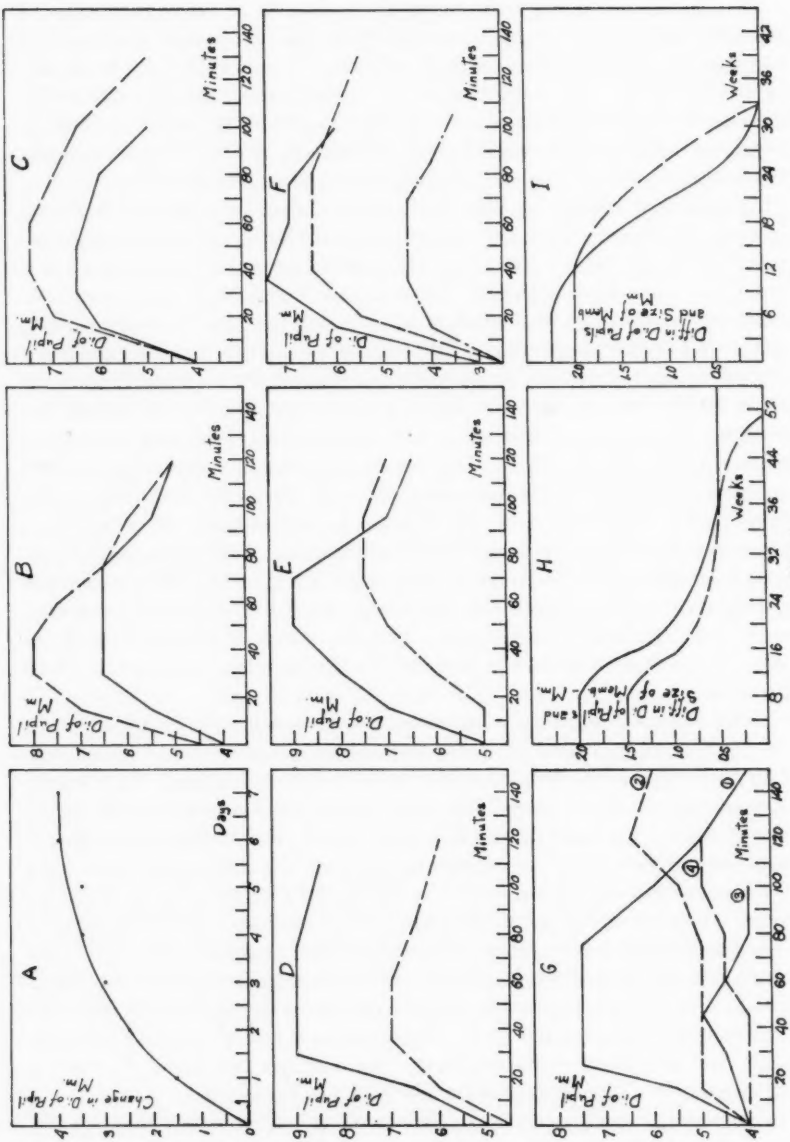
[3] This work was done in association with Dr. Gordon H. Scott.

Sensitization of smooth musculature by denervation. The sensitization of smooth musculature to the constricting action of epinephrine was first demonstrated by Meltzer and Auer (1912). Their results have been confirmed by others. The subject is reinvestigated here to obtain quantitative data as to the time order of the development of such sensitivity and its degree so that the results might serve as a basis of comparison in measuring the effect produced by other procedures instituted for this research.

As shown in figure 1A the time order of development of sensitivity plots as an exponential curve. Maximum sensitivity is attained in approximately 8 to 12 days. After this the sensitivity remains constant for a period of more than a year. Observations beyond this have not been made but there is no indication of any further increase or decrease with time. When the preganglionic fibers alone are interrupted the time order of development of sensitivity is similar but its final degree is approximately 10 to 20 per cent of that developed after removal of the postganglionic neuron. It is of some interest to estimate roughly the actual amount of sensitivity developed. Two cubic centimeters of epinephrine (1 to 1000) injected subcutaneously in the normal cats will produce generally not more than ½ mm. of dilatation of the pupils. In our animals, investigated in this research, 0.4 cc. of epinephrine usually caused a dilatation of the postganglionically denervated pupil averaging 2 to 4 mm. a 10 to 20 fold increase in sensitivity. The increase of sensitivity of the nictitating membrane, completely denervated by superior cervical ganglionectomy, is at least twice as great as that of the sympathetically denervated iris, i.e., 20 to 40 times.

Effect of hypophysectomy on sensitization to epinephrine. Animals with one pupil sensitized were subjected to total hypophysectomy. They were then tested for epinephrine sensitivity as before operation. The degree of sensitization of the sensitized pupil is increased approximately 40 to 50 per cent. The normal pupil is not sensitized sufficiently to show a response to threshold subcutaneous doses. The increased sensitivity develops within 4 to 6 hours and is nearly fully established in 24 to 48 hours. Frequently when in such animals the sensitized pupil was smaller than the normal before hypophysectomy, after recovery from the ether anesthetic the sensitized pupil was equal or increased in size over the normal. Such a change might remain permanently or it might decrease somewhat in three to ten days. Postoperative infections tend to exaggerate the sensitivity to epinephrine. An example typical of the results obtained in this type of experiment is shown in figure 1B.

Experiments were carried out in which the hypophysis was simply exposed without removal. In such animals there was no evidence of any increased sensitization. Hypophysectomized animals were frequently given large doses of anterior lobe extract, 5 cc. daily for 5 to 6 days adminis-


A
Change in D. of Rupt. Mm.
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B
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Minutes
C
D. of Rupt. Ms.
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Minutes
E
D. of Rupt. Mm.
Minutes
F
D. of Rupt. Ms.
Minutes
G
D. of Rupt. Mm.
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H
Diff. in D. of Rupt. and Size of Memb. - Mm.
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I
Diff. in D. of Rupts and Size of Memb. Ms.
Weeks

Fig. 1

tered intraperitoneally, with no change in the degree of sensitization. It is felt, therefore, that the loss of the posterior lobe hormone directly or indirectly is the cause of this sensitivity rather than that of the anterior lobe. Normal animals given 1 cc. of surgical pituitrin subcutaneously do not show any pupillary change. Hypophysectomized animals likewise show no change in the normal pupil but in the sensitized pupil there is a small but definite dilatation indicating that as a result of the hypophysectomy there is some increased sensitivity to the stimulatory action of posterior lobe hormone.

Effect of thyroid feeding on epinephrine response. Large doses of thyroid extract, 3 grams per day, were fed to six sensitized animals averaging 2.5 to 3 kgm. in weight. The evidence of hyperthyroidism in the animals was moderate restlessness, slight loss of weight and considerable increased excitement after epinephrine administration. After a feeding period of six to eight days the animals previously tested were retested for sensitivity to epinephrine (fig. 1C). The result showed a definitely increased sensitivity of the sensitized pupil averaging about 30–35 per cent in the animals studied. The normal pupil was not sensitized sufficiently by the thyroid to give evidence of increased sensitivity to subcutaneous epinephrine in amounts just over the threshold dose for the sensitized pupil.

Another group of normal animals was tested for pupillary response with 0.2 cc. of epinephrine intravenously and then fed 3 grams of thyroid per

Fig. 1. A. Graph indicating rate of development of sensitivity of pupil to epinephrine after sympathetic denervation. Dosage 0.4 cc. (1–1000) administered subcutaneously.

B. Graph indicating increased sensitivity of denervated pupil 8 days after hypophysectomy. Dosage 0.4 cc. subcutaneously.

C. Graph indicating increased sensitivity of denervated pupil 7 days after thyroid feeding. Three grams thyroid extract given daily for 7 days. Dosage 0.4 cc. subcutaneously.

D. Graph indicating decreased sensitivity of denervated pupil 6 months after total thyroidectomy. Dosage 0.4 cc. subcutaneously.

E. Graph indicating decreased sensitivity of denervated pupil 6 weeks after bilateral orchidectomy. Dosage 0.4 cc. subcutaneously.

F. Graphs indicating decreased sensitivity of denervated pupil 3 weeks after unilateral and 4 days after bilateral adrenalectomy. Ten cubic centimeters cortin (Upjohn) given daily after bilateral adrenalectomy. Dosage 0.4 cc. subcutaneously —normal, — after unilateral adrenalectomy, — after bilateral adrenalectomy.

G. Graphs indicating effect of activity on sensitivity of denervated pupil. Curves (1) and (2) cat 20. Curves (3) and (4) cat 19. Solid lines normal.

H. Graphs indicating time order of return of tone of pupil and nictitating membrane after postganglionic denervation. Solid line pupil, broken line nictitating membrane.

I. Graphs indicating time order of return of tone of pupil and nictitating membrane after preganglionic denervation. Solid line pupil, broken line nictitating membrane.

day for 7 days. At the end of that time they were similarly tested and it was found that the normal pupil was also more sensitive to epinephrine.

Effect of thyroidectomy on epinephrine response. In a series of 6 animals the thyroid glands were removed after a normal base line of sensitivity had been established. Results showed a gradually increasing diminution in sensitivity. This was well established after a week's time. The animals were followed up to a period of six months, with a final decrease in average sensitivity of 30 to 40 per cent (fig. 1D).

Effect of removal of ovaries and testes on epinephrine sensitivity. In a series of eight sensitized animals the testes or ovaries were removed. From time to time up to six months, postoperatively, such animals were tested as before operation. In a week's time it was evident (fig. 1E) that a diminution in sensitivity to epinephrine had developed in both sets of animals. After a month it approximated 30 to 35 per cent of that induced by denervation and thereafter remained more or less constant.

Effect of removal of one adrenal gland. After removal of one adrenal gland sensitized animals frequently showed a slight diminution in their sensitivity but in others the effect was almost negligible.

Effect of removal of two adrenals. After bilateral adrenalectomy with or without the administration of cortin the sensitivity of the test pupil was greatly diminished, 50 to 60 per cent (fig. 1E). This diminution was definitely established within 24 hours and lasted throughout the periods of observation, the longest of which was 8 days. In this series of experiments the diminution in sensitivity was checked by the intravenous route in order to eliminate possibility of the results being due to altered absorption from the subcutaneous tissues. With this method also the marked diminution in sensitivity was established. When larger doses of epinephrine were given the persistence of the sensitized state in the test pupil was readily established.

Concerning the specificity of the sensitization induced by denervation. When denervated blood vessels were tested for increased sensitivity to epinephrine by the method indicated above the results were definitely positive. These same blood vessels did not show any definite evidence of a similar increased sensitivity to pituitrin. The results of a typical experiment showing the differences in response of sensitized and normal blood vessels to epinephrine and pituitrin are shown in figure 2.

Effect of activity on the response of sensitized musculature to epinephrine. Animals with sensitized pupils were exposed to flashes of light to produce a contraction and dilatation of the pupils at a rate of 20 per minute for periods of 1 to 4 hours. When these animals were given test doses of epinephrine, it was found that their responses were delayed and in some instances somewhat lower than in the unstimulated state (fig. 1G). The possible significance of these findings is discussed below.

Cytological studies on sensitized smooth muscle. By none of the methods used was it possible to determine any definite and consistent change in the walls of denervated vessels followed up to 6 months after denervation. The incinerated preparations revealed no alterations in salt arrangements (fig. 3A). There was no measurable difference in spectrograms of treated

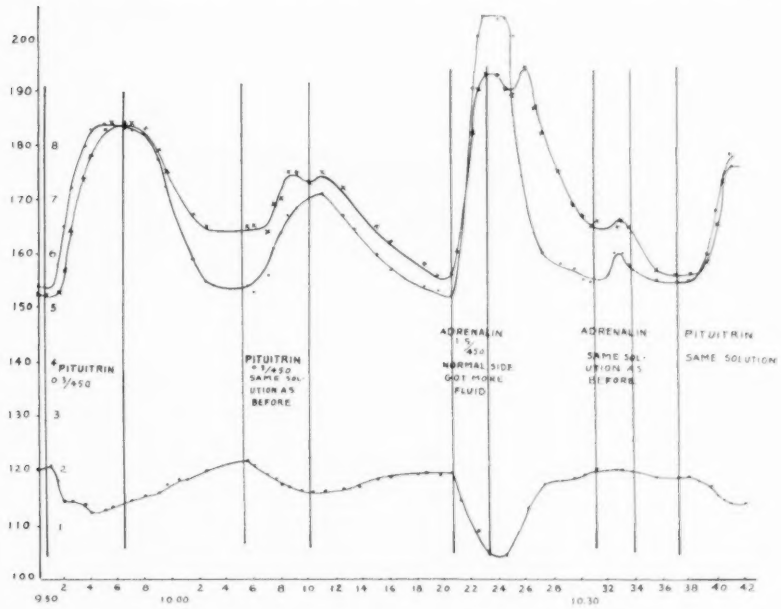

Fig. 2. Graphic record of bilateral perfusion experiment in rabbit in which the left femoral artery had been sympathectomized 9 months previously. Perfusion at equal rate on the two sides except for brief periods from 10.05 to 10.07 and from 10.21 to 10.25 when the flow on the right or normal side was 10 per cent faster than on the left. Abscissa—time in minutes. Ordinate—manometer pressure readings millimeters of mercury and rate of flow in drops per second. It will be noted that pituitrin (0.3 cc. in 450 cc. Ringer's) constricts the left side first but finally constricts both sides about equally. Generally the left side dilates first also. This is true even when right side flow is kept 10 per cent faster at 10.055. Epinephrine 1.5 cc. (1–1000) in 450 cc. Ringer's definitely constricts the left or sympathectomized side more than the right.

and control vessels (fig. 3B). The fat distribution was unchanged. It was found that sections stained by iron hematoxylin and hematoxylin and eosin were to all appearances identical with the normal, thus confirming Clark (1933). Fresh sections of vessels were also examined with polarized light and no differences found.

Relationship between sensitivity to epinephrine and smooth muscle tone.

Throughout this research many opportunities were provided for the investigation of a possible relationship between the degree of epinephrine sensitivity and smooth muscle tone following denervation of the preganglionic and post-ganglionic types. The observations were made on the nictating membrane and iris. It was evident that no relationship exists. The greatest loss of muscle tone occurs immediately after denervation. Epinephrine sensitivity is not maximal for from 6 to 12 days. Normal tone of postganglionically denervated smooth muscle is gradually reës-

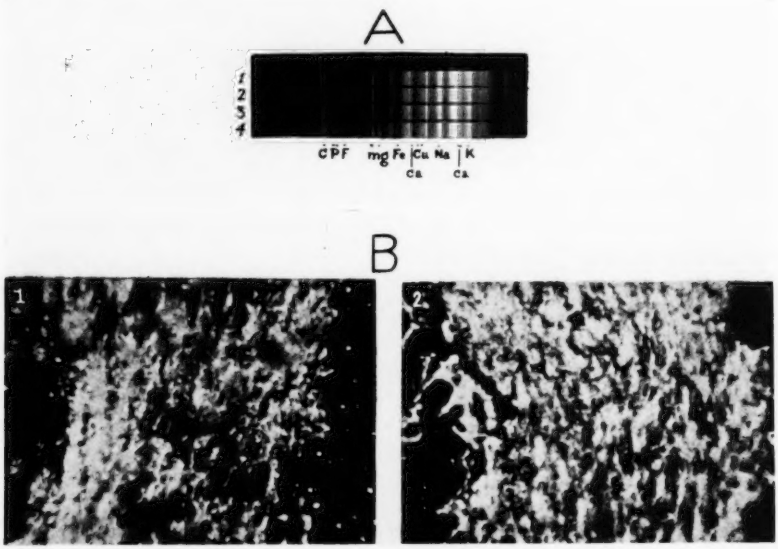

Fig. 3. A. Spark spectrograms of fresh femoral arteries of dog three months after sympathectomy of left one. 1 and 3 control right artery. 2 and 4 denervated left artery. Careful comparison of enlarged spectra fails to show any essential difference in the spectrograms.

B. Photographs of micro-incinerated preparations of dog arteries. 1. Normal femoral artery, right side. 2. Left femoral artery 3 months after denervation. They show no essential difference in salt concentration or arrangement.

tablished in 4 to 12 months so that at the end of this time the diameter of the denervated pupil and the degree of retraction of the nictitating membrane is equal to or nearly equal to that obtaining on the normal side (fig. 1H). Throughout the period of tone return there is no alteration of sensitivity to epinephrine. Preganglionically denervated tissue reëstablishes normal tone in approximately half the time required to reëstablish it in postganglionically denervated tissue (fig. 1I). Their epinephrine sensitivity remains unaltered during this period.

It has been shown above that the sensitivity of tissues is altered by the production of hormonal imbalance. Such imbalance may in itself produce some slight change in muscle tone but it is not sufficient or constant enough for analysis. Temporary diminution in smooth muscle tone may result during the postoperative period following hypophysectomy or bilateral adrenalectomy. In the hypophysectomized animals the sensitivity to epinephrine is increased at such times whereas in the adrenalectomized animal it is markedly decreased again indicating an absence of correlation between sensitivity to epinephrine and degree of smooth muscle tone. There is no persistent loss of smooth muscle tone from adrenalectomy.

Discussion. It is apparent from the observations made that denervation is the most effective method so far found for permanently sensitizing smooth muscle to epinephrine. Changes in such sensitivity induced by removal of endocrine glands, except possibly the removal of both adrenals, is slight in comparison. Indeed, none of the procedures which were found to alter the sensitivity of sensitized smooth muscle to epinephrine resulted in a sufficient alteration of such sensitivity in normally innervated smooth muscle to be demonstrable by the subcutaneous injection of epinephrine in ordinary doses. On intravenous injection it was possible to show that such altered sensitivity does effect normal tissues also but its degree is slight in comparison with what occurs in denervated tissues. It would appear that the effect of endocrine imbalance is augmented by the altered physiological state of denervated tissues.

The nature of the change responsible for sensitivity can only be speculated upon. If analogous to the mechanism of insulin sensitivity, it would be due to removal of factors normally counteracting the action of epinephrine. It may be the result of an altered trophic state of the tissues such as is doubtless present on the administration of thyroid extract. It has not been possible to demonstrate even with micro-incineration and spectography a physical basis to correspond with the altered physiological state of denervated smooth musculature. The assumption seems warranted that no inorganic salt or metal changes are responsible for the epinephrine sensitivity. If there is any change in lipoid content it is beyond the limit of our experimental methods to detect.

It was hoped that the experiments in which the denervated tissues were exercised before testing with epinephrine might be decisive in demonstrating a utilization of epinephrine or of cell constituent on which it acts because of the impression that the loss of functional activity resulting from denervation might be expected to lead to an accumulation of one or both of these elements. The experimental results do not justify the interpretation that such has been demonstrated. However they are suggestive of this and the marked diminution in sensitivity after bilateral adrenalectomy

is presumably due in whole or in part to a failure of replenishment of the epinephrine content normally present in tissue. Explanations based on an altered permeability have been offered by Cannon and Rosenblueth (1936) and others. These must be regarded as speculative, as the possibility exists that the action of epinephrine is a surface one.

In the present state of our knowledge it seems best to confine ourselves to the statement that as a result of denervation or of endocrine imbalance a trophic or metabolic change is effected in smooth musculature which makes it hyperdynamic or hypodynamic with reference to its response to epinephrine.

SUMMARY

Quantitative data on the sensitivity changes of denervated smooth musculature are presented. Such increased sensitivity is still further increased by thyroid feedings and by total hypophysectomy. To a lesser degree normally innervated smooth musculature is similarly affected.

The action of exogenous epinephrine on sensitized and presumably also on normal tissue is decreased by thyroidectomy, by gonadectomy and by removal of the adrenals.

Changes in sensitivity to epinephrine are not paralleled by changes in tone of denervated smooth musculature.

The change in tissues induced by denervation is specific in the sense that it permits an altered epinephrine response but not an altered response to pituitrin.

No physical or chemical basis for the altered physiological condition of denervated tissue has been demonstrable by micro-incineration, by spectrography or by routine cytological methods.

Evidence is presented not inconsistent with the interpretation that the increased sensitivity of smooth musculature to exogenous epinephrine may be due to an increased store of epinephrine or of a substance on which it acts.

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DISTRIBUTION IN BODY FLUIDS AND EXCRETION OF INGESTED AMMONIUM CHLORIDE, POTASSIUM CHLORIDE, AND SODIUM CHLORIDE

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This paper presents observations made during the first hours after oral administration of single doses of the salts. The changes observed in the composition of serum and urine have been correlated with the now accepted views concerning the composition and volume of the intracellular and extracellular body fluids.

These views may be summarized in part as follows: The extracellular fraction of the body fluid has approximately the composition of an ultrafiltrate of plasma, and in man contains most of the sodium and practically all of the chloride of the organism. The tissue cell membranes are ordinarily impermeable to the ions, Na^+, Mg^{++}, Cl^-, HPO_4^{--}, SO_4^{--}, and SCN^-, and to sucrose; consequently, after an intravenous injection of a non-isotonic solution of one of these substances, a new equilibrium is reached between extra- and intracellular concentrations, not through exchange of these solutes, but through a movement of water (Peters, 1935; Lavietes, Bourdillon and Klinghoffer, 1936; Bourdillon and Lavietes, 1936).

EXPERIMENTAL PROCEDURES. The same subject (J. B.) male, in good health, and weighing approximately 65 kilos, was used for all experiments, which were performed in the post-absorptive state. No food was taken during the procedure: water was administered with the salt in sufficient amounts to avoid nausea; some water was absorbed later in moderate amounts during the experiments, as recorded in the tables. No catharsis resulted.

The chemical procedures used were as follows (Peters and Van Slyke, 1932): CO_2 in serum and urine after Van Slyke and Neill; Cl in serum after Patterson, and in urine after Volhard-Harvey for experiments 1, 2 and 3; Cl in serum and urine after Van Slyke and Sendroy for the other experiments; sodium in serum and urine after Butler and Tuthill; potassium in urine after Shohl and Bennett for experiments 1, 2 and 3; potassium in

[1] Experiments 1, 2 and 3 reported in this paper were made in the Department of Internal Medicine, Yale University.

serum and urine after Kramer and Gittleman for the other experiments; ammonia in urine after Van Slyke and Cullen; urea in urine, with urease, after Van Slyke. Serum total base was determined after Hald (1933). The interstitial fluid volume was determined with CNS, as described previously (Lavietes, Bourdillon and Klinghoffer, 1936).

TABLE 1

Effect of NH_4Cl perorally administered

SERUM						URINE						
TIME	CO_2	Cl	Total base	Urea N	INTERSTITIAL FLUID VOLUME	TIME	Cl	Na	K	NH_3	Urea N	Volume	REMARKS
	m.Eq. per liter	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter**	*liters*		*m.Eq. per hour*	*m.Eq. per hour*	*m.Eq. per hour*	*m.Eq. per hour*	*m.Eq.* per hour*	*cc. per hour*	
9:15	28.8	99.9	149.1		19.2	8:00– 9:15							*Exp. 1.* 9:15–9:40 a.m.: 182 m.Eq. NH_4Cl + 700 cc. 1:00 p.m. 150 cc. coffee + light lunch
12:15	21.3	106.8	150.4		18.6	9:15–12:15	17.7	11.0	3.8	4.2†		138	
						12:15– 2:15	31.5	20.0	6.5	4.8†		158	
7:20	29.6	100.4			16.4								*Exp. 2.* 7:20–7:50 a.m.: 182 m.Eq. NH_4Cl + 600 cc. water 10:30, 150 cc. coffee
10:30	23.1	106.9			16.2	7:20–10:30	15.3	7.6	5.0	4.5†		175	
1:20	23.6	105.9			15.9	10:30– 1:20	21.7	10.8	8.8	3.9†		88	
8:15	27.0	100.5	144.5		18.0								*Exp. 3.* 8:15–8:45 a.m.: 182 m.Eq. NH_4Cl + 600 cc. water 11:30, 150 cc. coffee
11:15	23.3	106.4	145.2		17.9	8:15–11:15	16.2	9.7	3.5	3.7†		70	
						11:15–12:45	26.6	15.6	8.3	4.2†		333	
8:40	30.6	101.8		7.5		7:05– 8:40				1.8		30	*Exp. 4.* 8:45–9:35 a.m.: 187 m.Eq. NH_4Cl + 800 cc. water
11:30	23.2	108.4		10.1		8:40–11:30	15.5			5.2	44.0	247	
12:50	23.5	107.8		9.7		11:30–12:50	14.0			4.1	42.7	131	

* Expressed as m.Eq. of NH_4.

† These determinations were made by Dr. P. H. Lavietes.

Discussion of results. *Effects of NH_4Cl ingestion. Excretion.* That ammonia excretion was accelerated within the first three hours is shown by table 1. It is well known that after repeated NH_4Cl administration over periods of many hours or one or more days a great increase of ammonia output can occur (Gamble, Blackfan and Hamilton, 1925). It has been shown in this laboratory in experiments on dogs with explanted kidneys (Van Slyke, Rhoads, Hiller and Alving, unpublished) that am-

monia absorbed as NH_4Cl is so quickly changed to urea, that there is but very slight increase in the arterial blood ammonia, and that the ammonia excreted by the kidney is not at all the same ammonia absorbed, but is freshly formed in the kidney itself by the mechanism first discovered by Nash and Benedict (1921). If the same is true for man, it follows that the kidney responds to the acidosis with increased ammonia formation rather more quickly than had been supposed.

The water output during the experiments was approximately equal to the intake, so that there was no significant change in the fluid content of the body.

The rate of Cl excretion was such that in three hours after ingestion one quarter, or somewhat more, of the administered Cl was excreted.

Serum chloride. In table 2 the fourth column gives the Cl concentration increments theoretically calculated as $\frac{\text{Retained Cl}}{\text{Liters interstitial fluid}}$. The

TABLE 2

Calculated and observed changes in serum Cl and CO_2 after NH_4Cl administration

EXPERIMENT NUMBER	a RETAINED Cl CALCULATED AS INTAKE − OUTPUT	b INTERSTITAL FLUID VOLUME	$c = \frac{a}{b}$ CALCULATED + Δ Cl	OBSERVED − Δ CO_2	OBSERVED + Δ Cl	OBSERVED + Δ UREA
	m.Eq.	*liters*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*
1	129	19	6.8	7.5	6.9	
2	134	16	8.4	6.5	6.5	
	72	16	4.5	6.0	6.5	
3	133	18	7.4	3.7	5.9	
4	143	17*	8.4	7.4	6.6	2.6
	124	17*	7.3	7.1	6.0	2.2

* Assumed value.

assumptions on which the calculation is based are: 1. In the first 3 hours all the ingested Cl was absorbed from the gastrointestinal tract, so that the Cl retained in the body proper could be calculated as ingested Cl minus excreted Cl. 2. The retained Cl was distributed uniformly in the serum and other interstitial fluids. 3. None of the retained Cl penetrated the tissue cells (that entering the red blood cells and concentrated in the kidney is neglected). 4. The interstitial fluid volume was measured with sufficient accuracy by the sulfocyanate method.

The agreement between the fourth and sixth columns in table 1 indicates that the results are consistent with these assumptions.

Serum urea. It has been repeatedly shown (Peters and Van Slyke, 1931) that when NH_4Cl is ingested the ammonia is transformed into urea, and that urea in the higher animals diffuses freely into both intra- and extra-

cellular fluids, so that (except for the kidney) it is found nearly everywhere in the body in approximately the same concentration per unit of water present (Peters, 1935). Granting these premises, and that the interstitial fluids comprise about $\frac{1}{3}$ of the body fluids, one would expect that, after NH_4Cl ingestion, increase in urea concentration in all the fluids would be about $\frac{1}{3}$ the increase in Cl concentration, since the latter is confined to the interstitial fluids. Comparison of the last two columns of table 2 indicates that the serum urea increment was in fact about $\frac{1}{3}$ the Cl increment.

Serum CO_2. The agreement between the fifth and sixth columns of table 2 confirms Gamble and Ross (1923) in showing that the serum CO_2 decrease caused by NH_4Cl ingestion approximates the Cl increase. Since CO_2 changes within the limits noted are approximately (ca. 95 per cent) bicarbonate changes, near equality of Cl and CO_2 changes would be expected if the HCl, liberated by change of the NH_3 into urea, decomposes an equivalent of bicarbonate in the interstitial fluids.

Effects of KCl ingestion. Excretion. The striking fact evident in the right hand side of table 3 is that there was not only an increase in the excretion rates of K and Cl, which was expected; there were also marked, though short-lived, increases in the renal outputs of Na and CO_2 (the CO_2 representing in large part HCO_3). The outpouring of the latter ions began quickly; it also ended quickly, so that in the second urine, passed later than 2 hours after ingestion of the KCl, the rates of Na and HCO_3 excretion had fallen most of the way to their original levels.

The immediate effect of the KCl absorption was therefore to cause an excretion of all four of the chief plasma ions, Na, Cl, HCO_3 and K. In its apparent haste to get the total electrolyte concentration of the plasma down to normal, the kidney seemed to lose some of its usual discrimination; instead of eliminating only the K and Cl that had invaded the body, it also excreted Na and HCO_3 during the period immediately following the ingestion.

Serum electrolytes. The serum chloride increase in the experiments of table 3 (first two hours after KCl ingestion) was approximately what one would calculate on the assumption that the retained Cl was distributed uniformly in the plasma and other interstitial fluids (as after NH_4Cl ingestion) and also that there was no dilution of interstitial fluid. The serum K increase, however, was only about one-half as great. There was at the same time a decrease in serum CO_2.

Possible mechanism of the electrolyte changes following KCl ingestion. The sequence of events may be the following:

1. Leaving the gastrointestinal tract, K and Cl diffuse together into the blood and extracellular water. 2. The Cl stays in the extracellular fluids and does not penetrate the cells (except the erythrocytes) but the K distributes itself to the intracellular fluids as well. In consequence of

the wider distribution of the absorbed K, the increment of K concentration in the plasma is less than the Cl increment. 3. The K in diffusing

TABLE 3

Effect of KCl perorally administered

TIME	SERUM			INTERSTITIAL FLUID VOLUME	TIME	URINE						REMARKS
	CO_2	Cl	K			CO_2	Cl	Na	K	Urea N	Volume	
	m.Eq. per liter	*m.Eq. per liter*	*m.Eq. per liter*	*liters*		*m.Eq. per hour*	*m.Eq. per hour*	*m.Eq. per hour*	*m.Eq. per hour*	*mgm. per hour*	*cc. per hour*	
10:20	28.7	104.0	4.2	16.6	8:15–10:20	0.3	6.0	2.0			39	*Exp. 5.* 10:20–10:30: 161 m.Eq. KCl + 350 cc. water
12:05	26.2	108.0	6.4	16.5	10:20–12:05	8.6	23.4	12.0	23.6*		120	
					12:05– 1:20	2.0	24.8	4.8	25.7*		88	
10:20	30.7	102.6	4.7		9:15–10:20	0.7	5.5	2.3	3.6		50	*Exp. 6.* 10:25–10:35: 154 m.Eq. KCl + 350 cc. water 12:30; 250 cc. water + 15 gm. glucose
12:15	27.2	108.4	7.5		10:20–12:15	8.7	28.5	14.1	26.0	455	157	
					12:15– 1:55	2.0	28.4	5.5	24.8	387	96	
					1:55– 3:15	0.9	17.8	4.6	12.4		60	
8:30	30.0	103.8	5.6		(8:35–10:00)	(11.4)					(335)	*Exp. 7.* 8:35–9.05: 161 m.Eq. KCl + 500 cc. water 11:10; 150 cc. water + 15 gm. glucose
11:00	26.8	108.2	8.0		8:35–11:00	9.0	40.6	22.1	31.0		267	
12:05	26.7	106.8	6.5		11:00–12:05	0.1	24.9	3.7	23.5		83	

* As urine was not ashed, these figures should be slightly too high.

TABLE 4

Calculated and observed changes in serum Cl, K, and HCO_3 after KCl ingestion. First period of 2 to 3 hours after ingestion in each experiment

EXPERIMENT NUMBER	a RETAINED K AND Cl CALCULATED AS INTAKE—OUTPUT		b INTERSTITAL FLUID VOLUME	$c = \frac{a}{b}$ CALCULATED		OBSERVED		CALCULATED* $-\Delta HCO_3$ FROM URINE HCO_3 OUTPUT ALONE	OBSERVED	
	Cl	K		$+\Delta$ Cl	$+\Delta$ K	$+\Delta$ Cl	$+\Delta$ K		$-\Delta HCO_3$	Δ Cl $-\Delta$ K
	m.Eq.	*m.Eq.*	*liters*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*	*m.Eq. per liter*
5	120	120	16.5	7.3	7.3	4.0‡	2.2	0.9	2.5	1.8
6	99	104	(17)**	5.8	6.1	5.8	2.8	1.0	3.5	3.0
7	63	86	(17)**	3.7	5.1	4.4	2.4	1.3	3.2	2.0

* HCO_3 in this column is calculated as mM. CO_2.

** Assumed interstitial fluid volume, from average on this subject.

‡ The first period in experiment 5 covered only 1½ hours after KCl ingestion. The fact that observed serum Δ Cl was lower than calculated indicates that absorption in this period was probably not completed.

into the cells must be accompanied by some anion. The tissue cells appear to be impermeable to Cl but not to HCO_3 (Fenn, 1936). Hence it appears

probable that the K which passes into the tissue cells is accompanied by an equivalent of HCO_3.

If these were the only events influencing the three ions in the plasma, the plasma concentration changes, indicated as Δ values, could be expressed by the equation:

$$\Delta Cl - \Delta K = - \Delta HCO_3 \quad (1)$$

The left hand member, multiplied by the interstitial fluid volume, would represent the amount of absorbed K that has passed into the tissue cells, while the right hand member similarly multiplied would represent the HCO_3 accompanying the K into the cells.

If the observed values of table 4 are applied to the equation, the values for $-\Delta HCO_3$ calculated from the K and Cl changes are found to be from 0.5 to 1.2 m.Eq. smaller than $\Delta Cl - \Delta K$, indicating that, if only one equivalent of HCO_3 accompanied the K into the cells, some additional CO_2 left the interstitial fluids by another channel. This channel is indicated by the urinary CO_2 values of table 3 to be the kidneys. The amounts of CO_2 excreted in the second periods of experiments 5, 6 and 7 vary from 15 to 21 millimoles, which is from 0.9 to 1.3 millimoles per liter of interstitial fluid. Allowing for the fact that part of the CO_2 in the urine was free carbonic acid, only part in the form of HCO_3, the HCO_3 excretions appear to be close to the amount, 0.5 to 1.2 m.Eq. per liter interstitial fluid, which one must add to the right hand member of equation (1) in order to make the equation balance.

The above explanation is based on the assumptions that the tissue cells are permeable to K, and either to HCO_3 anions, or to CO_2 capable of conversion to HCO_3 within the cells. For these assumptions with regard to both HCO_3 and K the literature offers evidence.

With regard to HCO_3 Fenn (1936) has found that the muscle cells of the frog contain about 7 m.Eq. per kilo, and that carbonic acid added to blood in which frog muscle was suspended penetrated into the muscle cells.

With regard to potassium Fenn (1936) summarizes data showing that muscle cells are permeable to it, and that there is in the animal equilibrium between K in the muscle cells and K in the plasma, such that K passes back and forth between cells and plasma in response to changes in plasma K concentration and pH. In experiments where carbonic acid was added to frog muscle in frog blood, Fenn found that K was "driven from the blood into the muscle to neutralize the acid inside the muscle." Furthermore, evidence for the general penetration of retained K into the tissue cells is furnished by Norn (1929) in an injection experiment with a nephrectomized rabbit. His figures showed that the increase in serum K concentration was so low that the great part of the injected K must have left the extra-

cellular fluids, the only apparent path of exit being into the tissue cells. With Dr. I. H. Page we have confirmed this finding in an experiment as follows:

A 3000 gram rabbit was narcotized with amytal, and the ureters were ligated. Then 8 millimoles of KCl were injected intravenously with 50 cc. of water, during a period of 70 minutes. The K concentration of the serum rose from a pre-injection value of 2.4 m.Eq. to 6.6 m.Eq. per liter. To limit the rise to the observed 4.2 m.Eq., the injected 8 m.Eq. of K would have to be distributed uniformly in 1.9 liters of fluid, which corresponds closely to the total water content of a 3000 gram rabbit.

Concerning the hypothesis that retention of HCl is followed by migration of $KHCO_3$ into the tissue cells, it therefore appears from the above discussion that the migration of K may be assumed with fair certainty, and that migration of the HCO_3 is a possibility consistent with what is at present known. To make the evidence stronger, it would be desirable

TABLE 5

Effect of NaCl perorally administered

TIME	SERUM			TIME	URINE					REMARKS
	CO_2	Cl	Na		CO_2	Cl	Na	K	Volume	
	m.Eq. per liter	*m.Eq. per liter*	*m.Eq. per liter*		*m.Eq. per hour*	*m.Eq. per hour*	*m.Eq. per hour*	*m.Eq. per hour*	*cc. per hour*	
9:10	29.1	102.8	142.7							*Exp. 8.* 9:15-9:35:
12:05	28.6	108.4	147.1	9:10–12:05	0.2	14.9	7.3	10.3	53	210 m. Eq. NaCl
				12:05– 1:15	0.1	12.7	5.3	9.3	43	+ 400 cc. water
										12:05: 400 cc. water
										+ 20 gm. glucose

to perform with $KHCO_3$ animal experiments like that above outlined with injected KCl, and to analyze both plasma and tissues for CO_2. The necessity for special technique for accurate CO_2 analyses in tissues has prevented such experiments in connection with the work here reported.

Without tissue bicarbonate data, the possibility remains that the decrease in serum bicarbonate might have been due to acid decomposition of HCO_3. Such an occurrence would follow only if the KCl retention accelerated acid production by the organism.

If our interpretation of the results is correct, potassium chloride administration must increase the HCO_3, and in consequence the pH, within the tissue cells, as it does in the urine, while at the same time it decreases HCO_3 and pH in the extracellular fluids. There would result from KCl retention the curious phenomenon of a shift toward acidosis in the extracellular fluid, and toward alkalosis within the cells, whereas the effect of NH_4Cl would be only acidosis in the extracellular fluid.

Effects of sodium chloride ingestion. Finally, in table 5, a similar experiment with NaCl ingestion is given for comparison. This time a marked water retention is evident. There is no increase of bicarbonate excretion, nor appreciable change in serum CO_2. Cl is excreted only in part as NaCl; part passes out as KCl. The Na and Cl increases in serum each approximate 5 m.Eq.

If the interstitial fluid had remained constant at 17 liters the Cl and Na retained would have raised their concentrations in plasma by 10.6 m.Eq. However, in this experiment interstitial fluid measurements indicated an increase of 700 cc., which was the volume of water retained. This would have decreased the Cl and Na concentrations of the second serum sample by 4 per cent, or 4 and 6 m.Eq. respectively. It appears that the 700 cc. of water retained remained in the interstitial fluids, and prevented about half of the increase in Na and Cl concentration in those fluids that would otherwise have been caused by the NaCl retention.

SUMMARY

Single large doses of NH_4Cl, KCl and NaCl were administered by mouth to a human subject, and the changes in serum electrolytes, interstitial fluid volume, and electrolyte excretion were observed for several hours. These changes suggest the following conclusions:

1. The increment in serum Cl concentration following the administration of each salt approximates that estimated by assuming that the retained Cl diffuses into the interstitial fluid only, and does not enter the tissue cells.

2. The increment in serum urea concentration following NH_4Cl administration approximates that estimated by assuming that the retained N is entirely changed to urea, which diffuses into the total body fluids, both intra- and extracellular.

3. The increment in serum K concentration after KCl administration approximates that estimated by assuming that the retained K, like urea, diffuses into the total body fluids.

4. As regards the behavior of serum bicarbonate, it appears that:

After NH_4Cl administration it was reduced by acid decomposition.

After KCl administration, it was also reduced. About $\frac{1}{3}$ of the fall in serum HCO_3 was accounted for by excretion of bicarbonate in the urine. For the other $\frac{2}{3}$ the probable exit was by way of diffusion as $KHCO_3$ into the tissue cells, although the possibility of HCO_3 decomposition by accelerated acid formation in the body is not excluded.

After NaCl administration, serum bicarbonate was not appreciably affected.

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THE INFLUENCE OF DESTRUCTION OF THE ADRENAL MEDULLA ON EMOTIONAL HYPERGLYCEMIA IN RATS

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Cannon, Britton, Silvette and Britton, Lumley and Nice, and others have presented evidence that the presence of the adrenal medulla is essential for the experimental production of emotional hyperglycemia. The criticism which is frequently advanced against evidence for a change in the physiologic response of the demedullated animal to "emergency" situations assumes that the experimental destruction of the medulla also involves destruction of a critical amount of cortical tissue. In the present study we have compared normal rats and rats whose adrenal medulla has been destroyed in respect to changes in blood sugar in response to fright produced by loud sounds. We believe that there has been no deficiency in the function of the adrenal cortex in any of our animals.

METHODS. The adrenal medulla was destroyed by two different procedures: The first experimental group of animals was prepared by the transplant method in which the adrenal bodies of adult female rats are transplanted to the ovaries. As described by one of us (Ingle) and Higgins, there is early degeneration of the entire medulla and most of the cortex down to a surviving capsule and layer of glomerulosa cells. Regeneration occurs from the capsule and the restored graft contains approximately a normal mass of cortical tissue but is entirely free of medulla. The second group of animals was prepared by the enucleation method described by Evans. The capsule of each adrenal was opened and the nucleus of the gland, which contains the entire medulla, was expressed by pressure. Regeneration of a normal mass of cortical tissue takes place from the remaining capsule.

Fifty adult female rats were closely matched into pairs on the basis of age and body weight. In one animal of each pair the transplantation procedure was carried out. These animals were treated with cortin during the first ten days following operation. Twelve similar animals were matched into pairs and one animal of each pair was subjected to bilateral enucleation of the adrenal glands. These animals were not treated with cortin. A minimal period of forty days was allowed between the time of operation and the beginning of the experimental study. All animals survived this period of delay in good health.

A wooden box, 24 inches (61 cm.) square, was divided by a heavy wired-glass partition. Animals were placed on one side of this partition and two packages of firecrackers of the flash variety were exploded on the other, the animals thus being protected from burns and noxious fumes.

For each experiment the following procedure was uniform: After a fasting period of twenty-four hours, samples of blood were drawn from the tail of a demedullated animal and from its matched control. Both animals were then excited and samples of blood were again drawn at approximate intervals of five minutes. Determinations of blood sugar were made by a modified Folin-Wu technic (Gibson, 1930) by an experimenter who was unaware which were the control animals and which the animals that had been operated on.

TABLE 1

Average changes in blood sugar in rats after emotional excitation

	BLOOD SUGAR IN UNSTIMULATED RATS		BLOOD SUGAR IN RATS FOLLOWING EXCITATION					
			1-10 minutes		11-20 minutes		21-03 minutes	
	Number	Mgm./ per cent	Number	Mgm./ per cent	Number	Mgm./ per cent	Number	Mgm./ per cent
Demedullated	22	94 73	19	81 42	21	82 08	19	81 66
S. D.		22 44		23 09		26 15		21 84
Normal	21	93 52	19	101 95	21	112 98	21	115 19
S. D.		19 67		14 33		20 90		22 29
Difference		1 21		20 53		30 90		33 53
Diff./S. D. Diff.		0 18		3 21		4.13		4.68

After completion of these studies on emotional hyperglycemia, fifteen rats were taken at random from the group with transplanted glands and their glycemic response to a subcutaneous injection of 0.25 cc. of a 1:5000 solution of epinephrine chloride was determined.

RESULTS. The average values for the blood sugar of the animals whose adrenal glands had been transplanted and for their normal controls at various periods after excitation are summarized in table 1. The average value prior to excitation is approximately equal in the two groups. Within ten minutes after excitation there was an increase in the blood sugar for the normal animals and a decrease for those in the transplant group. During the next twenty minutes the level of the blood sugar in animals in the transplant group showed little change, whereas that in the normal animals continued to rise.

Comparable results were obtained for the animals whose adrenal glands had been enucleated. There was a drop below the initial level of 16 to 19 mgm. per 100 cc. whereas their normal controls showed rises of 37 to 47

mgm. per 100 cc. It is significant to note that all normal animals showed a rise in blood sugar and all of the enucleated group of animals a fall.

Fifteen animals with transplanted adrenal glands were given injections of epinephrine, and an average maximal rise of 40 mgm. per 100 cc. was observed subsequent to injection.

Necropsy was performed on all the animals used in these experiments. Each animal was found to possess an approximately normal mass of viable cortical tissue.

COMMENT. These experiments support the conclusion that the presence of the adrenal medulla is essential for the manifestation of emotional hyperglycemia in the rat. The decrease in blood sugar which was observed after excitation in those animals lacking the medulla may be due to an artefact. It was suggested to us by Doctor Keys that since the determinations of blood sugar were made on whole blood, and since the sugar content of cells is lower than that of serum, the increase in percentage of cells in the blood which commonly follows emotional excitement would have the effect of lowering the average values of the determinations made on whole blood.

We believe that there has been no deficiency in the function of the adrenal cortex in the animals studied in these experiments. Previous studies (Evans; Ingle; Ingle and Harris) on animals prepared by these methods have given evidence to support this contention. It has been further shown in the present experiment that our animals were capable of showing an increase in blood sugar in response to epinephrine when it was injected.

SUMMARY

Normal rats show an increase in the level of blood sugar in response to emotional excitation, whereas rats whose adrenal medulla has been destroyed by either the enucleation or transplant method show a decrease in blood sugar under identical experimental conditions. Animals whose adrenal medulla has been destroyed show a marked increase in the level of blood sugar when epinephrine is injected.

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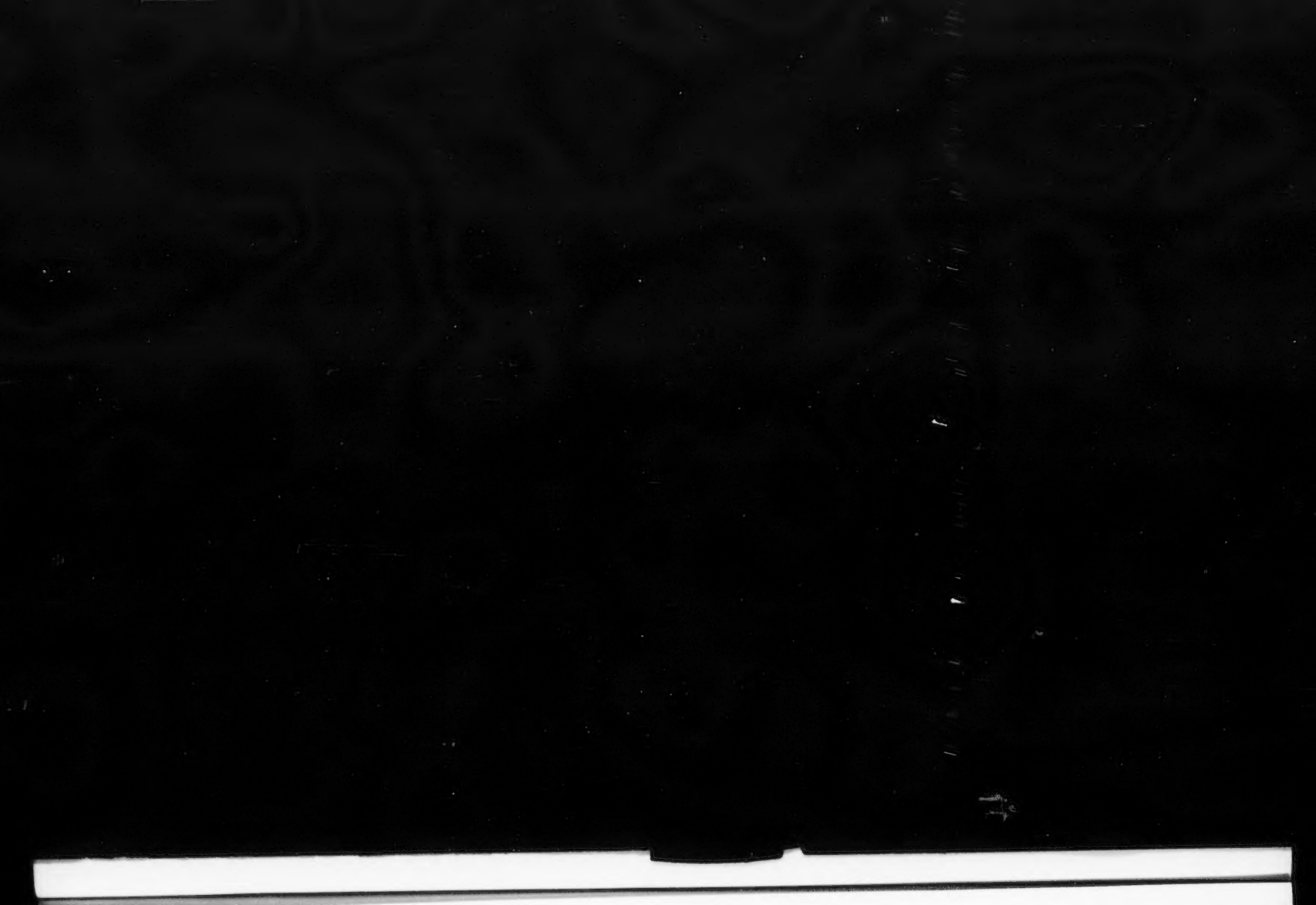
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